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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

Studies on the Elimination of Strychnine . . 289
Lloyd W. Hazleton and Frank Fortunato

A Study of a Strychnos Species 295
F. V. Lofgren and Dale L. Kinsley

Method of Evaluating Relative Efficacy of Disguising Agents for Distasteful Drugs . 298
W. Arthur Purdum

The Pharmacology of Sodium Hydroxyacetate with Observations on the Toxicity of Glycine 306
Walter F. Riker and Harry Gold

Estimation of Magnesium in Solution of Citrate of Magnesia 312
Frederick K. Bell

Celastrol, Spectrographic Characterization and Color Tests 315
L. F. Fieser and R. N. Jones

U. S. Pharmacopœial Convention Statement of Income and Expense 317

Books Received 320

Abstracts 289–320

Advertising—Cover Pages 2, 3, 4, and pp. I to VIII

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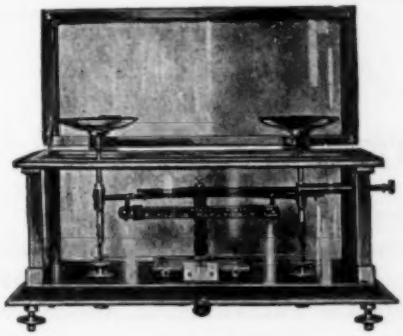

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(1) Doe, J. B., *Am. J. Physiol.*, 79 (1927), 289.

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Scientific Edition

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Studies on the Elimination of Strychnine*

By Lloyd W. Hazleton and Frank Fortunato

The problem of the disposition of strychnine by the animal body has been the subject of several previous studies. Hale (1), using extraction with immiscible solvents and a colorimetric test, came to the conclusion that strychnine is slowly excreted and that dogs develop a slow, imperfect tolerance, while results from guinea pigs are less conclusive. Hatcher and Smith (2), using more than lethal doses in anesthetized dogs and estimating biologically in cats, found that small amounts of strychnine were excreted in the urine within a few minutes, and Hatcher and Eggleston (3) noted no tolerance in cats, dogs or guinea pigs, following chronic studies of 12 days' duration. Evidence presented in the above papers indicates that urinary excretion of strychnine accounts for only a portion of the total administered, that active diuresis increases the amount excreted but is not a life-saving measure, and that the liver plays a part in the detoxification of strychnine. In humans, Weiss and Hatcher (4) found that the liver eliminates about $^{5}/_{6}$ of the total and the kidneys excrete only that which the liver fails to eliminate. It has been reported by Mostrom and McGuigan (5) that repeated injections of strychnine nitrate shorten the onset of spasms in frogs and they conclude that a "habit" is formed by use of pathways which are not normally used. There was apparently no tolerance.

Following subcutaneous administration to white rats, Schwartze (6) found that these animals could all dispose of 33% of the lethal dose within 2 hrs., while half of them could dispose of 50% and an occasional animal could dispose of 67% during the same period. He also pointed out that all evidence of strychninization disappeared within 18 hrs. and that intermittent injections (at 2-hr. intervals) produced a discontinuous or uneven strychninization.

Taking into consideration the fact that analysis of excreta for small, tolerable amounts of strychnine is very difficult and that such values at their optimum represent only a portion of the ingested drug, the studies herein reported have been directed toward (*a*) pharmacological detection of the accumulation of strychnine, or of tolerance to it, following prolonged periods of daily administration, and (*b*) determination of the rate of essential elimination or duration of pharmacological action. The applications and limitations of essential elimination

* Received August 14, 1942, from the Henry E. Kalusowski Memorial Laboratory, George Washington University School of Pharmacy, Washington, D. C.

Acknowledgment is made of a grant from the Proprietary Association in support of this study.

TABLE I.—THE EFFECT OF DAILY PARENTERAL ADMINISTRATION OF ONE-HALF THE CONVULSIVE DOSE OF STRYCHNINE ON THE SLEEPING TIME OF RATS FOLLOWING INTRAPERITONEAL INJECTION OF SODIUM PENTOBARBITAL

No. of Rats	Sex	Daily Strychnine, Days	Pentobarbital, Mg./Kg.	Sleeping Time, Min. Mean	Sleeping Time, Min. Range	Mean Weight, Gm.	Date
7	*F*	Parent	30	141	134–150	232	May 12
7	*F*	Control	30	84	71–93	138	Apr. 28
10	*F*	20	30	90	79–103	127	Apr. 29
9	*F*	Control	30	222	169–279	224	Sept. 4
7	*F*	145	30	204	183–265	211	Sept. 4
8	*M*	Parent	30	59	47–67	331	May 12
10	*M*	Control	30	45	30–62	173	Apr. 28
9	*M*	20	30	49	44–60	183	Apr. 29
9	*M*	Control	30	67	46–87	323	Sept. 6
7	*M*	147	30	68	16–102	321	Sept. 4

studies have been discussed by Dille (7) and by Hazleton and Green (8).

In order to avoid the possibility of variation in the potency of the drug such as Ward, *et al.* (9), reported, all of the drug was administered in the form of solutions of U. S. P. Strychnine Sulfate from a single lot.

EXPERIMENTAL

DAILY PARENTERAL ADMINISTRATION

Rats.—Male and female adult, albino rats on a diet of Purina dog chow checkers were used in these experiments. The use of adults only was deemed advisable in view of the observations of Schwartze (10) and of Poe, *et al.* (11), that the toxicity of strychnine decreases with the age of the rat until maturity, after which it stabilizes. The experiments consisted of two groups as follows:

1. Following the determination of the approximate convulsive dose for females as 1.25 mg./Kg.,[1] one-half this dose was given daily by intraperitoneal injection to a group of 10 animals, a comparable number of controls being kept under identical conditions. The rats were weighed and the daily dose adjusted at approximately 30-day intervals throughout the entire period of 205 days. At appropriate intervals the response of both the treated group and the controls to light anesthetic doses of sodium pentobarbital and to the convulsive dose of strychnine was determined.

The convulsive dose for males was determined as 2.0 mg. and the experimental procedure outlined above was followed for a group of 10 males and their controls. The period of daily administration for this group was 185 days. By the term "daily" is meant 6 days per week.

(*A*) The results obtained following the intraperitoneal administration of 30 mg. of sodium pentobarbital 24 hrs. after the last daily dose of strychnine are shown in Table I. Sleeping time was measured from the time of injection until the animals were able to right themselves after being gently turned onto their backs. Statistical evaluation by Fisher's table of "t" method (12) indicates that there is no significant difference between the controls and the treated animals of any experiment. While the effect of strychnine on barbiturate narcosis is debatable, it is significant that daily administration of strychnine does not alter in any way the normal response to this typical central nervous system depressant. In the female group, it was noted during the final experiment that after the tail and front quarters made definite righting movements, there was an abnormally prolonged delay before the hind quarters recovered sufficiently to permit complete righting. Since righting was taken as the end point, this phenomenon may account in part for the marked difference in sleeping time between this group after 20 days and after 145 days. A portion of the difference in sleeping time for both males and females may also be attributed to age and seasonal variation. In connection with the latter factor there are included in Table I values for males and females of the untreated parent generation which were determined in the spring of the year at a time comparable to the 20-day determination. For each sex this value falls between the 20-day value (spring) and the final value determined in the fall when the experimental groups were comparable in weight with the parent generation.

(*B*) At various intervals the animals described above were used to determine the response to the convulsive dose of strychnine 24 hrs. after the last daily dose. The results from the females are summarized in Table II; from the males, in Table III. Although there is considerable variation in both sexes it would appear that (*a*) there was no accumulation of the drug since, in spite of a low margin of safety between the convulsive and lethal doses, the deaths were infrequent and uniformly distributed, and (*b*) any tendency toward tolerance is extremely slight and does not appear in either sex until after 54 days of administration. During the interval between the 165th and the 205th day, the rats of the control group and of the treated group were mated and daily administration of strychnine was continued throughout mating and pregnancy. Approximately 10 days post partum the values for the 205th day

[1] Unless otherwise noted all doses are expressed in milligrams of drug per kilogram of animal body weight. Hereafter the words "per kilogram" may be omitted to avoid repetition.

were obtained and the series discontinued. Both the controls and treated groups appear to be slightly more susceptible to strychnine following pregnancy.

TABLE II.—THE RESPONSE OF FEMALE RATS TO INTRAPERITONEAL INJECTION OF A CONVULSIVE DOSE OF STRYCHNINE FOLLOWING VARIOUS PERIODS OF DAILY PARENTERAL ADMINISTRATION OF ONE-HALF THE CONVULSIVE DOSE. TWENTY-FOUR HOURS ELAPSED BETWEEN THE LAST DAILY DOSE AND THE CONVULSIVE DOSE

No. of Rats	Daily Strychnine, Days	Dose of Strychnine, Mg./Kg.	Convulsions Positive	Negative	Death
6	Test dose	2.0	6	0	6
4	Test dose	1.5	4	0	3
12	Test dose	1.25	7	5	0
10	Test dose	1.125	1	9	1
9	Control	1.25	5	4	0
9	30	1.25	9	0	0
9	Control	1.25	8	1	0
10	54	1.25	7	3	1
9	Control	1.25	6	3	0
.7[a]	165	1.25	2	5	0
9[b]	Control	1.25	9	0	0
5	205	1.25	4	1	0

[a] Two animals were killed by error in daily dose.
[b] These are post partum values; two with litters not included.

TABLE III.—THE RESPONSE OF MALE RATS TO INTRAPERITONEAL INJECTION OF A CONVULSIVE DOSE OF STRYCHNINE FOLLOWING VARIOUS PERIODS OF DAILY PARENTERAL ADMINISTRATION OF ONE-HALF THE CONVULSIVE DOSE. TWENTY-FOUR HOURS ELAPSED BETWEEN THE LAST DAILY DOSE AND THE CONVULSIVE DOSE

No. of Rats	Daily Strychnine, Days	Dose of Strychnine, Mg./Kg.	Convulsions Positive	Negative	Deaths
12	Test dose	2.0	4	8	2
10	Control	2.0	2	8	0
10	25	2.0	3	7	1
10	Control	2.0	4	6	1
9	54	2.0	4	5	1
9	Control	2.0	4	5	1
6	167	2.0	2	4	0
8	Control	2.0	6	2	0
7	185	2.0	3	4	1

2. The convulsive dose for metrazol in female rats was determined as 35–38 mg. following intraperitoneal injection. The 38-mg. dose was adopted for the following experiments: To determine the additive effect of strychnine and metrazol, 0.1 convulsive dose of strychnine was injected intraperitoneally and 10 min. later, at about the time of maximum absorption, the convulsive dose of metrazol was given. The results in Table IV indicate that there is no additive effect from these doses, or from the combination of 0.25 convulsive dose of strychnine with the convulsive dose of metrazol under similar conditions. There is, however, a slight increase in the time of onset of convulsions, measured from the injection of the metrazol.

One-half the convulsive dose of strychnine was given daily to a group of 17 female rats for a period of 30 days, and 24 hrs. after the last daily injection, the convulsive dose of metrazol was administered. There was a slight increase in the number of convulsions and a rather marked delay in the time of onset. After 7 additional days of strychnine injections, metrazol produced a marked increase in incidence of convulsions with a decrease in time of onset, although the latter remained above the normal. Four days after the withdrawal of strychnine, the incidence of convulsions following metrazol was still high and the time of onset prolonged. Even after 25 days' withdrawal the response was about twice normal, although after this period the latent period had returned to normal. Following this period the response to the convulsive dose of strychnine was approximately normal.

The results of the experiments just described indicate that while no additive effect between single doses of strychnine and metrazol could be demonstrated, the prolonged daily administration of strychnine sensitizes or conditions the animals to the convulsive action of metrazol.

Monkey.—An adult, male rhesus monkey was used in these experiments. The diet consisted of Purina dog chow checkers supplemented by half an orange daily, water *ad lib.* and occasional greens. The convulsive dose of strychnine following sub-

TABLE IV.—THE RESPONSE OF FEMALE RATS TO THE INTRAPERITONEAL INJECTION OF A CONVULSIVE DOSE OF METRAZOL FOLLOWING SINGLE INJECTIONS OF STRYCHNINE AND FOLLOWING VARIOUS PERIODS OF DAILY ADMINISTRATION OF ONE-HALF THE CONVULSIVE DOSE OF STRYCHNINE

No. of Rats	Single Dose of Strychnine, % of C. D.	Daily Strychnine, Days	Metrazol, Mg./Kg.	Convulsions Occurrence Number	Occurrence Per Cent	Time of Onset, Min.
27	...	...	35	10	37	3.5
29	...	...	38	11	38	3.5
14	10	...	38	6	43	4.2
14	25	...	38	6	43	3.7
17	...	30	38	9	53	5.7
17	...	37	38	13	76	4.3
17	...	Withdrawn 4 days	38	15	88	4.7
17	...	Withdrawn 25 days	38	14[a]	82	3.6
15	100	Withdrawn 31 days	..	10	67	...

[a] Two deaths, the first of the series.

cutaneous injection was found to be 0.6 mg. This dose caused hyperexcitability, twitches of the limbs, incoördination and an occasional mild, spontaneous, clonic convulsion followed by recovery. At this time the body weight was 3.25 Kg., and daily subcutaneous administration of one-half the convulsive dose, or 1.0 mg. total, of strychnine was begun. This dosage was continued for 220 days (except Sundays), at the end of which period the body weight was 4.63 Kg. and daily dosage represented 36% of the convulsive dose. Twenty-four hours after the last daily dose, the convulsive dose of 0.6 mg. was repeated and the response was almost identical with the original. Daily administration was discontinued. These results are indicative of no accumulation or change in susceptibility to the action of strychnine after a prolonged period of daily administration of the drug.

After a period of 85 days during which no drug was administered, the above dose of 0.6 mg. was repeated. The response was more marked than previously, as was also the response to the same dose again 2 weeks later, each case necessitating the administration of sodium pentobarbital to insure complete safety of the animal. At intervals of two weeks, single doses of 0.5 and 0.4 mg. were given. The smaller dose caused only hyperexcitability, slight incoördination and an occasional twitch, while the dose of 0.5 mg. duplicated very closely the effects formerly produced by 0.6 mg. and was taken as the convulsive dose for later experiments on essential elimination.

ESSENTIAL ELIMINATION

Rats.—The rate of essential elimination of strychnine by male and female rats was determined by intraperitoneal injection of portions of the predetermined convulsive dose at definite intervals of time until the onset of convulsions. Details of the method and the importance of the various factors involved have been discussed elsewhere (8), and it will suffice here to say that the results shown in Table V indicate that when the rate of administration is sufficiently rapid female rats can essentially eliminate strychnine at a rate of 0.9 mg./Kg./hr., which is equal to 72% of the convulsive dose per hr. Pregnant females have a lower rate of essential elimination, the average for ten animals being 0.67 mg./Kg./hr., which is equal to 54% of the convulsive dose for nonpregnant animals per hr. This lower rate is presumably due to the abnormal *central nervous system/body weight* ratio and perhaps to some extent to the apparent increased susceptibility of pregnant animals noted in the post partum results above.

The average rate of essential elimination for male rats was 1.85 mg./Kg./hr., or 93% of the convulsive dose per hr., although some showed practically no elimination and others had a considerably higher rate. This extreme variation in the response of male rats to strychnine has been noted by other workers, notably Ward and Crabtree (13). In the table those animals which convulsed when a total of only one convulsive dose had been given are indicated as less than (<) the value for the next injection, while those that did not convulse after two or more convulsive doses are indicated as more than (>) the value for the last dose given.

Ten minutes is the shortest practical interval, since this approximates the average time of onset of convulsions following the single doses administered to the animals in Tables II and III. The rate of essential elimination was calculated according to the formula:

$$\frac{\left(\begin{matrix}\text{Total drug in}\\ \text{mg./Kg.}\end{matrix}\right) - \left(\begin{matrix}\text{Convulsive dose}\\ \text{in mg./Kg.}\end{matrix}\right)}{(\text{Time in hrs.})} = \left(\begin{matrix}\text{Essential elimination}\\ \text{in mg./Kg./hr.}\end{matrix}\right)$$

Time is measured from the time of the first injection to the time of the last injection, no allowance being made for the interval between the injection and the convulsion since this latent period should be approximately equivalent to that following the single injection of a convulsive dose.

Rabbits.—In preliminary experiments it was found that intravenous injection of 0.17 mg. caused convulsions in 4 of 6 animals, with 2 deaths, and this dose was considered to be the convulsive dose for the basis of the following experiments.

Adult male albino rabbits were used and the rate of essential elimination was determined by two methods, the first of which was by intravenous injection of portions of the convulsive dose at definite

TABLE V.—THE RATE OF ESSENTIAL ELIMINATION OF STRYCHNINE BY RATS FOLLOWING INTRAPERITONEAL INJECTION OF PORTIONS OF THE CONVULSIVE DOSE AT VARIOUS INTERVALS OF TIME. CONVULSIVE DOSE (C. D.) = 1.25 MG./KG. FOR FEMALES, 2.0 MG./KG. FOR MALES

No. of Rats	Sex	Rate of Administration C. D., %	Interval, Min.	Average Rate of Essential Elimination Mg./Kg./Hr.	C. D./Hr.	No. of Injections, Av.	Remarks
5	*F*	25	15	>0.65	>0.52	11	No convulsions
5	*F*	25	15	0.65	0.52	7.4	
10	*F*	25	10	0.67	0.54	6	Pregnant
10	*F*	25	10	0.90	0.72	7	
10	*M*	25	20	>0.94	>0.47	9	No convulsions
2	*M*	25	15	1.13	0.57	8	
7	*M*	25	15	>1.13	>0.57	10	No convulsions
4	*M*	25	10	<0.80	<0.40	4	Value of fifth
9	*M*	25	10	1.85	0.93	9	
6	*M*	25	10	>2.28	<1.14	14	No convulsions

TABLE VI.—THE RATE OF ESSENTIAL ELIMINATION OF STRYCHNINE BY RABBITS FOLLOWING INTRAVENOUS INJECTION OF PORTIONS OF THE CONVULSIVE DOSE AT VARIOUS INTERVALS OF TIME. CONVULSIVE DOSE (C. D.) = 0.17 MG./KG.

No. of Rabbits	Rate of Administration C. D., %	Interval, Min.	Average Rate of Essential Elimination Mg./Kg./Hr.	C. D./Hr.	No. of Injections, Av.	Remarks
5	10	15	> 0.012	> 0.07	12	No convulsions
4	20	15	> 0.082	> 0.48	11	No convulsions
10	20	10	0.122	0.72	12	All convulsed
3	20	8	0.146	0.86	11	All convulsed
4	20	5	0.183	1.08	8.5	All convulsed

TABLE VII.—RATE OF ESSENTIAL ELIMINATION OF STRYCHNINE IN RABBITS FOLLOWING SLOW INTRAVENOUS INFUSION OF SOLUTION CONTAINING 0.02 MG./CC. CONVULSIVE DOSE (C. D.) = 0.17 MG./KG. FIGURES IN PARENTHESES ARE CORRECTED VALUES BASED ON A 3-MIN. LATENT PERIOD

Wt. of Rabbit, Kg.	Vol. of Solution, Cc.	Total Strychnine, Mg.	Elapsed Time, Min.	Rate of Administration Cc./Min.	Rate of Administration Mg./Kg./Hr.	Average Rate of Essential Elimination Mg./Kg./Hr.	Average Rate of Essential Elimination C. D./Hr.
2.41	54.1	1.08	112	0.48	0.241	0.149	0.88
	(52.6)	(1.05)	(109)			(0.147)	(0.86)
2.60	65.5	1.31	77	0.85	0.393	0.261	1.53
	(62.9)	(1.26)	(74)			(0.255)	(1.50)
2.02	27.2	0.54	30	0.91	0.539	0.200	1.18
	(24.6)	(0.49)	(27)			(0.164)	(0.96)
2.40	55.8	1.12	60	0.93	0.465	0.295	1.74
	(53.0)	(1.06)	(57)			(0.286)	(1.68)
2.07	60.9	1.22	43	1.42	0.829	0.585	3.44
	(56.7)	(1.14)	(40)			(0.562)	(3.31)

intervals of time until convulsions occurred. Calculation of the rate was the same as that described for rats above. The results are summarized in Table VI and it will be noted that the rate of administration considerably influences the determined rate of essential elimination. Except for the first group of animals, each injection represented 0.2 convulsive dose or 0.034 mg./Kg., the final rate of administration being determined by the frequency of injection. Since intravenous administration eliminates the absorption factor, it is necessary to consider only the latent period of action to establish the minimum interval of time which will allow full development of effects before the succeeding dose is given. The latent period for strychnine sulfate following intravenous injection to rabbits was found to be 3 or 4 min. and therefore a 5-min. interval should allow for unusual variation in the response of individual animals. At this rate of administration rabbits can essentially eliminate an average of 0.183 mg./Kg./hr., or slightly over one convulsive dose per hr.

The second method of determination involved the slow intravenous infusion of a dilute solution of strychnine sulfate. The animals were tied to the operating position in a supine position and, under local anesthesia with 2% procaine hydrochloride and epinephrine, the external jugular vein was exposed and cannulated. In some of the experiments, respiratory and blood pressure tracings were made from the cannulated trachea and common carotid artery, respectively. Although these tracings aided in differentiating between the occasional struggles and the strychnine convulsions, they are not essential to the experiment.

The strychnine solution, containing 0.02 mg./cc., was administered from a burette at various manually controlled rates until definite spontaneous convulsions occurred. From the data thus obtained the rate of administration and the rate of essential elimination were calculated and are presented in Table VII. The corrected data are based on a latent period of 3 min. for the onset of convulsions following intravenous injection of strychnine. It will be noted that the rate of essential elimination increases with the rate of administration with the exception of the third animal in the table. In this case the convulsion was relatively mild and the infusion was continued for 12 min. longer before the incidence of a violent convulsion which terminated in death. At this time the rate of essential elimination was 0.253 mg./Kg./hr. The results from these experiments, unqualified, indicate that following sufficiently rapid intravenous infusion rabbits can essentially eliminate at least 0.562 mg./Kg./hr., or about three convulsive doses per hr.

Monkey.—In the experiments previously described, the latent period between subcutaneous injection of strychnine and the onset of convulsions in the monkey was 10 to 12 min. and the convulsive dose, 0.5 mg. For the determination of the rate of essential elimination by injection of portions of the convulsive dose at definite intervals, 0.2 convulsive dose was injected every 15 min. until the occurrence of convulsions after the seventh injection. The rate of essential elimination, calculated as previously described, was thus 0.127 mg./Kg./hr., or approximately 25% of the convulsive dose per hr.

Guinea Pigs.—In view of the previously mentioned inconclusive results from guinea pigs it seemed advisable to include this species in the current investigations. Attempts to establish a mean convulsive dose for strychnine, however, led to the

conclusion that methods based on such a value could yield valid results only if extremely large numbers of animals were used. Inspection of Table VIII will show that nonlethal convulsions can be obtained with doses of from 5 to 10 mg., the double integrated data (14) indicating a mean value of approximately 7 mg. As a result of the above observations, no further attempt was made to determine the rate of essential elimination in guinea pigs.

TABLE VIII.—DETERMINATION OF THE CONVULSIVE DOSE OF STRYCHNINE SULFATE FOLLOWING INTRAPERITONEAL INJECTION IN GUINEA PIGS

No. of Animals	Dose of Strychnine, Mg./Kg.	Convulsions: Observed Data Positive	Convulsions: Observed Data Negative	Convulsions: Integrated Data Positive	Convulsions: Integrated Data Negative	Deaths
3	2.5	0	3	0	32	0
2	3.0	0	2	0	29	0
1	4.0	0	1	0	27	0
12	5.0	3	9	3	26	1
10	6.0	2	8	5	17	0
7	7.0	2	5	7	9	0
11	10.0	7	4	14	4	4
1	15.0	1	0	15	0	1
1	20.0	1	0	16	0	1
1	25.0	1	0	17	0	1

DISCUSSION

Throughout the course of these experiments there has been little evidence to indicate that the daily administration of strychnine to rats or the monkey over long periods of time has led either to accumulation or to the development of tolerance to the action of the drug. It must therefore be assumed that the total amount given has been essentially eliminated and that such prolonged administration does not interfere with the ability of the body to eliminate the drug. It is apparent, however, that pregnancy induces a relative increase in susceptibility and decrease in capacity for essential elimination and this fact may be of considerable importance if large doses of strychnine are to be given repeatedly.

If, as it appears, the daily administration of strychnine sensitizes the rat to the convulsive action of metrazol while single doses do not, it is desirable to consider the mechanism of such an action. Perhaps the observation of Mostrom and McGuigan (5) offers the best explanation until further information is available. Thus, while metrazol is not primarily a spinal drug, it does have some such action and if the synaptic resistance is gradually lowered by strychnine the spinal action of metrazol might be sensitized. Since evidence is cited to indicate that the strychnine does not accumulate and the time interval of this experiment would not include seasonal variation, it follows that the sensitization must be of physiological nature. Further support of this view is (*a*) in general, the time of onset of metrazol convulsions was decreased, (*b*) the sensitization was slow in developing and lasted for a considerable time after withdrawal of the strychnine, (*c*) the response to full doses of strychnine remains approximately normal. It is not probable that the sensitization is due to the accumulation of metrazol in view of the rapid rate of essential elimination reported by Hildebrandt and Muegge (15) and the possibility of acquired refractiveness to this drug discussed by Pollock, *et al.* (16).

The importance of maintaining a high blood concentration of drug for the study of elimination has been pointed out by Schwartze (6) for strychnine and by Haggard and Greenberg (17) for alcohol. The results herein described clearly demonstrate that a wide range of values for essential elimination may be obtained depending upon the method used and the rate of administration. In the infusion method, an apparent discrepancy exists between results indicating an essential elimination rate of 0.562 mg./Kg./hr. and the fact that administration of the drug at the rate of 0.241 mg./Kg./hr. will cause convulsions within 2 hrs. This is very largely due, however, to the influence of the rate of administration upon the rates of elimination and accumulation and is the subject of detailed consideration elsewhere (8). It is apparent that the intravenous infusion method will produce the higher results, but that extravascular repeated injection, while introducing the factor of variable absorption, will give valid and satisfactory values if the experiment is properly designed. The results from the latter method perhaps more nearly represent elimination of therapeutically administered drug than do the intravenous values.

Although Hatcher and Brody (18) discussed the significance of the latent period of action for digitalis in the estimation of its persistence of action, this factor has usually been ignored in the consideration of the elimination of other drugs. The correction for latent period of strychnine indicates that at slow rates of administration this procedure does not materially affect the results, but at more rapid rates the effect is significant and would be even more so if the latent period were relatively longer.

CONCLUSIONS

1. No definite evidence of accumulation or tolerance could be found following relatively long periods of daily parenteral administration of one-half the convulsive dose of strychnine to the rat or monkey.

2. Daily administration of the above dose of strychnine sensitizes the rat to the convulsive action of metrazol. A possible mechanism for this action is discussed.

3. Under the conditions described the following rates of essential elimination of strychnine sulfate were determined:

(*a*) Female rats, intraperitoneal, 0.9 mg./Kg./hr.

(*b*) Pregnant rats, intraperitoneal, 0.67 mg./Kg./hr.

(*c*) Male rats, intraperitoneal, 1.85 mg./Kg./hr.

(*d*) Male rabbits, repeated intravenous injection, 0.183 mg./Kg./hr.

(*e*) Male rabbits, intravenous infusion, 0.562 mg./Kg./hr.

(*f*) Male monkey, subcutaneous, 0.127 mg./Kg./hr.

(*g*) Male guinea pigs, intraperitoneal, found unsatisfactory for the methods used.

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A Study of a Strychnos Species*†

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The genus *Strychnos* occupies a rather unique position in the vegetable kingdom because its various species exhibit widely varying physiological actions. While some of the species thus far examined from a chemical and physiological standpoint are exceedingly poisonous, others are entirely harmless, and in numerous cases highly prized as foods. The poisonous action is due mainly to the alkaloids strychnine and brucine. These alkaloids have been found to exist in all the different parts of some species. In other species, however, only certain parts are poisonous, thus indicating that the alkaloids may be localized.

In 1928 a quarantined plant of the species *Strychnos spinosa* was received from the U. S. Bureau of Plant Industry to be planted in the botanical garden at the University of Florida. The growth of this plant was not successful although it was able to exist. Upon inquiry at the Sub-Tropical Experiment Station at Homestead, Florida, it was learned that this species was thriving and producing fruits. Interest was aroused as to the alkaloidal content and toxicity.

After making a careful review of the literature it was found that the only investigations of this species had been on the fruits and even then conflicting results were reported concerning the toxicity and the presence of alkaloids. Also, through the years, the true identity of this plant had become confused.

This investigation was undertaken in the light of these reports to attempt to clear up these contradictions and uncertainties and

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to investigate the entire plant pharmacognostically.

HISTORY

Flacourt (1), in 1661, is the first to give an account of this plant which he called *Caniram vontac*. He states that the plant is also known as *Cydonium bengalense*. This according to an illustration of Plukenett (2) is not *Strychnos spinosa*.

Plukenett (2), in 1670, before the adoption of the binomial system of nomenclature, gives the name *Cucurbitifera arbor spinosa, fructu male aurei facie, aromatica*.

In 1791, Lamarck (3) gave this plant its present name of *Strychnos spinosa*. He calls this species synonymous with Flacourt's *Caniram vontac* and Plukenett's *Cucurbitifera arbor spinosa*. Poiret (1), in 1804, gave a complete description of this plant and called it *Vomique epineuse*. In 1842, Harvey (4) gave it the name *Brehmia spinosa*.

SCIENTIFIC NAMES AND SYNONYMS

The plant has several scientific names. As mentioned, *Strychnos spinosa* and *Brehmia spinosa* are said to be identical. "Index kewensis" (5) gives *Strychnos spinosa*, *Strychnos vuntac* and *Strychnos madagascariensis* as synonymous. The latter is misleading because Poiret (1) describes a strychnos species growing in Madagascar and calls it *Strychnos madagascariensis*. Yet, according to Poiret's description, it is characteristically different from *Strychnos spinosa*. Other names reported are *Vulgairment abre a Savonette* (1), *Strychnos ramis spinosa* (3) and *Vontaca Flacourt* (6).

Synonyms commonly ascribed to *Strychnos spinosa* are Kafir orange and Natal orange. Others are Vuntac (7), Calabash (8), Litonga seed (9), spiny spinosa (10), Vouavountaka (11) and Voavotaka (12). Native African synonyms are Klapper, Zulu um Hlala and Tonga Usuala (13).

REPORTED TOXICITY

The consensus is that the pulp of the fruit is edible, being described by most investigators as delicious. The seeds, on the other hand, have produced several contradictions as to their toxicity, three workers (14, 15, 16) stating that they are toxic and six investigators (5, 8, 17, 18, 19, 20) saying that they are nonpoisonous. Gilg (21) and Tunmann and Rosenthaler (22) seem to correlate these two views as to toxic effects when they state that the fruits are sometimes poisonous, sometimes nonpoisonous. Interesting is Small's (15) statement in which he says, "The seeds of the Kafir orange are so poisonous that great care must be exercised in eating the pulp in which the seeds are imbedded, in order to eliminate all seeds," in contrast to Flükiger's (7) statement, "they are nontoxic—in fact harmless."

The references which give their views negatively all agree that the seeds contain no alkaloids, while those investigators giving positive conclusions do not state definitely the causative substance giving such action. Perhaps the reasons for these two views may be answered by Gilg (21) when he infers that the toxicity is due to a bitter principle and not to an alkaloid.

Another possible answer to these contradictory opinions is the doubt which exists as to the real identity of the species. Those persons who have examined the fruits obtained their specimens from Africa. Gilg raises this doubt when he writes that "...the true *Strychnos spinosa* is indigenous only to Madagascar and that the species of the African mainland which we have designated up to now by that name belongs to a different very well defined characterized species."

There has been no experimental work on the toxicity of the leaves, stems and shell of the fruit (pericarp) reported in the literature.

EXPERIMENTAL

Fruits, stems and leaves of *Strychnos spinosa* were obtained from the Agricultural Experiment Station, Department of Agriculture, University of Florida, Homestead, Florida. A small quantity of fruit was also obtained from De Soto City (Highlands County), Florida. Two shipments of fruit were received from Homestead, one containing immature fruits, the other ripe fruits.

The stems received were divided between the last year's growth which was green in color, and the older stems, which had a whitish gray color.

Preparation of Material.—The seeds were removed from the pulp of the fruit and dried in an oven at a temperature of 37° C., as were the shells of the fruit, leaves and stems.

The hardness of the dried seeds caused extreme difficulty in getting a representative powdered sample. The ground samples that were obtained were the result of numerous grindings and regrindings with power and hand mills.

TABLE I.—RESULTS OF THE DETERMINATION OF ALKALOIDS IN *Strychnos spinosa*

Part Used	Method		
	U. S. P.	Stas–Otto	Continuous Extraction
Seeds of mature fruits	Negative	Negative	Negative
Seeds of immature fruits	Negative	Negative	Negative
Shells (pericarp)	Negative	Negative	Negative
Leaves	Negative	Negative	Negative
Stems (old)	Negative	Negative	Negative
Stems (new)	Negative	Negative	Negative

Examination for Presence of Alkaloids.—Three methods for extracting alkaloids were used, *i. e.*, (*a*) the U. S. P. method for nux vomica, using chloroform and ether as the extractive, (*b*) the Stas–Otto method and (*c*) a continuous extraction (Soxhlet) using alcohol acidulated with tartaric acid. Table I gives the results of the examinations for the presence of alkaloids.

Toxicological Studies.—Mice were put on a diet of the ground seeds. Some were given the unextracted seeds and some were given the marc of extracted seeds. No deaths occured in six days.

In order to assure that a sufficient dose was taken at one time and to determine if other parts of the plant were toxic, capsules were made up of the powdered material of the different parts of the plant. These capsules were then administered to guinea pigs. One-gram doses were given. After four hours the guinea pigs had shown no ill effects or any noticeable change.

An extract was made of the powdered seeds. This extract was concentrated so that 1 Gm. of the dried extract was equal to 10 Gm. of the powdered seeds. One-gram doses of the extract were administered to guinea pigs. No ill effects or change in reaction was observed.

Taxonomic Study.—In order to be sure that our present species growing in southern Florida was the true *Strychnos spinosa*, it was thought that a taxonomic comparison should be made between our sample and the descriptions given by earlier botanists.

Specimens were obtained from Homestead, Florida, and sent to the Division of Plant Exploration and Introduction, Division of Plant Industry, U. S. Department of Agriculture, Washington, D. C., to be examined. They replied that they could not give a critical determination of the plant because there is not a good collection of herbarium specimens of African plants in this country, and that their determination had to be based on literature. They also replied that the specimen sent did not compare with the original *Strychnos spinosa* specimen which was introduced in 1903 (14) and that there probably had been a transposition of labels somewhere through the ensuing years, or that this specimen was from another introduction. However, after studying the specimen, they could see no reason which would lead them to doubt that the plant was the true *Strychnos spinosa*.

The material sent to Washington has been preserved and placed in the Herbarium of the U. S. National Arboretum (Sheet No. 74,479). This will give a means for any future worker to check the identity of the material discussed in this paper and thus precludes the risk of this work becoming valueless if the identification is found to be incorrect.

Histology.—The histology of the plant parts, including cross sections of the seed, shell, leaf and stem, was studied for the purpose of identification. Also the diagnostic elements in the powdered material of each of these plant parts were observed and described. The results of these studies are embodied in a thesis which is on record in the library of the University of Florida.

CONCLUSIONS

From the qualitative chemical examinations for the presence of alkaloids in *Strychnos spinosa* growing in Florida, it has been found that alkaloids are absent in the seeds, shell, stems and leaves. It is possible that an examination of the roots of this species might reveal the presence of alkaloids, although it is highly improbable. It must be remembered also that this plant is a native of Madagascar and tropical Africa and that a change in environmental conditions might not be conducive to the formation of alkaloids in the plant, which have been reported by some authors.

Toxicological examination of the seeds, shell of the fruit, stems (old and new) and leaves on guinea pigs and mice has shown *Strychnos spinosa* to be nontoxic.

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Method of Evaluating Relative Efficacy of Disguising Agents for Distasteful Drugs*

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During the long era of medical ignorance and superstition, it was common belief that medicines should be repulsive in taste or malodorous in order to drive the "evil humours" from the body. The public of today, as a whole, is not so ignorant of medical matters as were its ancestors even as little as a century ago. This has been brought about to a large extent by health education in the public schools, news accounts of the latest discoveries in pharmaceutical chemistry and pharmacodynamics, feature articles by physicians and men in allied professions appearing in newspapers and magazines, and by public lectures and radio addresses. The combined effect has been to make the public conscious of the fact that a dose of medicine need not be bitter, acrid, or nauseating, in order to be efficacious. Consequently, the physician who can prescribe effective therapeutic agents in palatable forms is more certain of success than one who has no regard for palatability. Disagreeable tasting substances are not relished by persons in good health and surely they should not be inflicted on ailing persons (for whom nausea and vomiting are not uncommon), if it is possible to avoid them.

Medicines which are administered in solid form present no serious problem with respect to taste. Provided they are not too bulky, they may easily be given in the form of cachets, capsules, coated pills or coated tablets, in which the taste of the medicament is entirely concealed. On the other hand, infants and most small children, as well as some adults, are unable to swallow such forms of medication, particularly cachets and large capsules. When such is the case, a liquid preparation must be given. Moreover, drugs in the liquid state are more quickly absorbed and thereby exhibit their therapeutic effects more promptly than solid forms of medication. Furthermore, there are a great number of drugs which are more conveniently dispensed in the liquid state. Many of these liquids are quite distasteful, solutions of iodides and bromides being notable examples.

This study was undertaken in an effort to devise a method for appraising the value of pharmaceutical vehicles as disguising agents for distasteful drugs and then to use the method for evaluating some of the more common vehicles as agents to disguise certain unsavory medicaments. A search of available literature revealed that very little has been done in this direction. Many vehicles have been recommended for distasteful drugs, but in the majority of cases there has been no obvious scientific basis for these recommendations.

Since the prime purpose of a vehicle is to lessen or completely overcome the disagreeableness of the medicament, it is rational to assume that the threshold of taste of the medicament varies directly with the disguising power of the vehicle employed,

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i. e., the higher the masking power of the vehicle, the higher the threshold value of the drug in that vehicle. If then threshold values are determined for a drug in a number of vehicles, that vehicle in which the drug exhibits the highest liminal concentration is the most efficacious of the agents tested.

Various techniques have been employed by different investigators in the determination of taste limens. Komuro (1) sprinkled the tongue with from 200 to 500 cc. of a solution at a definite temperature and, as a control, repeated this procedure using tap water at the same temperature. Kionka and Strätz (2) administered to their subjects from 6 to 10 cc. of solution which was washed back and forth in the mouth. These last named experimenters started with hyperthreshold concentrations and gradually decreased the strength of the solutions until the liminal strengths were reached, the solutions being maintained at body temperature and administered by means of a graduated pipette with a dropping attachment. Salmon and Blakeslee (3) found that 0.6 cc. was an adequate quantity to administer for a single test and began their determinations with solutions of subliminal concentrations, each successive solution being twice the strength of that which preceded. Ward, Munch and their associates (4, 5, 6) have determined strychnine thresholds, employing the technique developed by Munch (7) for the biological assay of capsicum. This method consists of comparing the pungency of an alcoholic extract of capsicum with that of a standard solution of piperine. Salt thresholds have been determined by Richter and MacLean (8) using dilute solutions of sodium chloride. After experimenting with several procedures, they conclude that the method which follows is the most satisfactory. Each subject started with a solution of hypothreshold strength and tasted successively stronger solutions until the threshold of saltiness was reached. Each concentration tasted was compared with distilled water and the subjects were instructed to taste each solution as often as necessary in order to reach a decision. Some of the findings of these workers were used to advantage in the development of the procedure described in the experimental section which follows.

EXPERIMENTAL

Throughout the experiments which follow, care was taken to maintain as nearly uniform conditions as was practicable. To avoid the possibility that the small amount of salts present in city water might interfere with the threshold determinations, only freshly distilled water was used in the preparation of solutions and for rinsing purposes between tests.

The chemicals employed were of U. S. P. or N. F. quality. Some of the pharmaceutical preparations were made in the laboratory according to U. S. P. or N. F. directions and others, for reason of convenience, were purchased from one of the large pharmaceutical manufacturers.

Glass-stoppered bottles were adopted for the storage of solutions after it was found that some of the more alert tasters could detect what they described as a "mustiness" in the distilled water and in some of the very weak aqueous solutions which had been kept in bottles with cork stoppers.

The distilled water and all solutions were stored at room temperature which varied from 25° to 28° C. While Komuro (1) has shown that threshold values obtained at 10° C. are much higher than those at 20° C., he also demonstrated that the threshold values were not markedly lower at 40° C. than at 20° C. Salmon and Blakeslee (3) made threshold determinations on a number of persons at about 8° C., at room temperature and at about 55° C. and observed no variation in the results at these appreciably different temperatures. In view of the above findings, it was not deemed necessary to store the solutions in an incubator at body temperature as did Kionka and Strätz (2). Although it is true that the solutions having a temperature of about 26° C. will produce a feebly cool sensation in the mouth, this temperature sensation should not interfere with the tasting of solutions since the control solutions used were also at the same temperature.

In a further effort to maintain uniform conditions all persons participating in the tests were requested not to eat within two hours prior to a threshold determination and the tobacco smokers were asked not to smoke within one-half hour before a test.

All subjects were cautioned to wash all samples of solutions and controls back and forth in their mouths so that these samples came in contact with all regions of their tongues and oral cavities. This agitation was essential since it has been shown by Öhrwall (9) that the various parts of the tongue's surface do not respond equally to the several qualities of taste.

In the ordinary determination of taste thresholds of aqueous solutions, the subject simply has to decide whether the solution in question has taste or is tasteless. However, with vehicles other than water, the problem is not so simple. It is necessary

for the taster to determine whether the medicinal solution has a taste exactly like or different from that of the plain vehicle used as the control.

Numerous preliminary trials were made before a satisfactory method for the determination of the threshold of taste of drugs in vehicles was found. While these preliminary experiments will not be discussed in detail, all findings of value are given in the paragraphs which follow.

It was found that if a small portion of solution was followed by a somewhat larger sample of control at the same temperature, the control seemed cooler to some of the subjects because it was capable of absorbing more heat from the mouth. This necessitated the administration of equal quantities of solutions and controls. Five cc. of each was arbitrarily chosen for use in subsequent experiments.

The use of full-strength syrups and elixirs as vehicles for the drugs proved unsatisfactory because, in the case of syrup of glycyrrhiza, its flavor so persisted that no reasonable number of washings with distilled water would entirely remove it from the mouth. In addition, the intensity of its taste seemed to increase, *i. e.*, the taste successively grew stronger as more samples were tasted. This cumulative effect also was reflected in the ability of the subjects to perceive the taste of the dissolved drug. As the taste of the syrup became more intense, a proportionately greater concentration of drug was required before its taste could be detected. Following this difficulty, all vehicular solutions and their corresponding controls were made to contain 10% by volume of the syrup or elixir in distilled water. The subjects reported that the flavors of the diluted vehicles did not persist and that they were rather easily removed by rinsing.

Kionka and Strätz (2) state that contradictory results have been obtained in the determination of taste limens due to advance knowledge on the part of the taster of the composition or strength of solutions to be tasted. In an effort to prevent the entrance of this psychological factor of self-deception into the results, no set order with respect to strength was followed in the administration of solutions in the early trials. This procedure was impractical because of the large number of solutions which had to be tasted with the resultant consumption of a considerable amount of time in order to make a single determination. Thereafter, the subject began with a solution of hypothreshold concentration and tasted successively stronger solutions in the series until the threshold strength had been reached. Self-deception, in all probability, is not an important factor in this work because Salmon and Blakeslee (3) have demonstrated that the threshold of a single subject varies enormously at different times and they state that extreme refinement in technique is, therefore, unnecessary.

At first, the concentration of distasteful drug in adjacent solutions in a series varied as the ratio 1 : 1.25. The subjects frequently experienced difficulty in distinguishing between solutions next to each other in a series so, in later tests, the ratio was increased to 1 : 2. This change simplified proper identification and effected a considerable saving of time in that fewer solutions had to be tasted in order to make a determination. The individuals who tasted these solutions remarked, in most instances, that the threshold concentrations were quite sharply defined. Naturally, the threshold values obtained were somewhat higher than those got formerly. From this, it was reasoned that if the ratio of strength of contiguous solutions was reduced to a point between the two already tried, greater accuracy could be attained than by use of the higher ratio without the uncertainty and confusion of opinion which resulted from use of the lower ratio. Extensive trials were made employing a series of solutions in which the concentration of drug in adjacent members varied as the ratio 1 : 1.5 and this was found to be quite satisfactory in all respects.

It became apparent that as the subjects gained experience in tasting, their acuity of perception increased for a time with a consequent lowering of threshold values. This obstacle was overcome by determining for each subject the threshold concentration of an aqueous solution of the drug immediately before each determination in a vehicular solution.

THE METHOD AND ITS APPLICATION

(*A*) *Comparison of Efficacy of 19 Official Vehicles in Disguising the Saltiness of Ammonium Chloride.*—Thirty-two adults served as subjects for these tests. It was desirable to have a minimum of 30 persons for the tests since it has been shown (10) that the accuracy of results obtained in any animal experimentation increases with the number of animals used. This is particularly true when small groups (numbering under 30) are used. When more than 30 animals are employed, the reliability of results is not increased sufficiently to warrant the time and expense of the additional determinations.

Ammonium chloride served as the distasteful drug in these experiments by reasons of its disagreeably salty taste and its wide usage as a medicinal agent. Ten concentrations of the salt ranging from 0.0044 *N* up to 0.17 *N* were used. As explained previously, a ratio of 1 : 1.5 existed between the strengths of adjacent solutions. The strength and corresponding number of each solution were as follows:

Normality	Number	Normality	Number
0.0044	1	0.033	6
0.0067	2	0.050	7
0.010	3	0.075	8
0.015	4	0.11	9
0.022	5	0.17	10

All vehicular solutions and controls contained 10% by volume of the vehicle (syrup or elixir).

Portions of 5 cc. of each strength of aqueous solution, each strength of vehicular solution, and of control, were placed in test tubes and the tubes, in turn, were placed in properly numbered racks.

On each working day, the limen for a subject was determined first in distilled water solution and then in the vehicular solution prepared for that day. The subject rinsed his mouth with distilled water and then tasted the aqueous solutions in ascending numerical order, rinsing between each solution tasted, until he reached the first solution which possessed a salty taste. Beginning one number below the number at which the threshold was reached in aqueous solution, the subject then tasted the vehicular solutions in ascending numerical order, following each with a control and rinsing before proceeding with the next solution. This was continued until the vehicular solution gave a sensation of saltiness when compared with the control. If a question arose on the part of a subject regarding the taste of one of the solutions, he was instructed to sample another portion of the same solution and if there was still doubt, to taste the solution next higher in concentration and to record this as his threshold if the taste was definite. No solutions were swallowed; all were ejected from the mouth after a decision was reached by the subject.

The difference between the aqueous and the vehicular thresholds is a measure of the disguising value of a particular vehicle. Even though the subjects develop a keener sense of taste during the progress of the experiments or if only the normal fluctuations in taste acuity occur, the mean difference should remain approximately the same; *e. g.*, if a person's threshold in aqueous solution appeared at concentration No. 4, and in a certain vehicle at No. 6, and upon repeating the test after an interval of several weeks, the numbers were 2 and 4, respectively, the difference in either case is 2. Proof of this theory is given in the next series of experiments (part *B*) in which quinine hydrochloride replaced ammonium chloride.

Since the object of this method is to establish the relative differences between the threshold values in the aqueous solutions and in the vehicular solutions, the concentrations at which the thresholds are reached simply provide the data for arriving at these differences and bear no other significance. Because of this and since the normalities represented by any two consecutive numbers in a series of solutions are proportional to those for any other pair of consecutively numbered solutions, the mean differences are expressed by numerals rather than by actual concentrations in terms of normality in Table I. These numerical differences will hereafter be referred to as "disguising potentials." The vehicles listed in Table I appear in the order of decreasing mean disguising potentials.

The standard errors for each set of determinations were computed by use of the formula $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, in which d represents the deviation of an individual determination from the mean, regardless of whether it is plus or minus, Σd^2 represents the sum of these deviations squared, and n is the number of determinations in the series.

To determine whether the mean threshold values for aqueous and vehicular solutions really differ significantly or whether these differences are due

TABLE I.—AMMONIUM CHLORIDE DISGUISING EFFICACY OF 19 DILUTED VEHICLES[a]

Vehicle	Number of Subjects Tested	Mean Threshold in Water ± Standard Error, $m_2 \pm \epsilon_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm \epsilon_1$	Mean Disguising Potential ± Standard Error, $D \pm \epsilon$	Significance of Difference[b]
Syrup of glycyrrhiza	32	3.56 ± 0.22	5.72 ± 0.29	2.16 ± 0.24	5.97
Syrup of raspberry	32	3.91 ± 0.21	5.72 ± 0.23	1.81 ± 0.20	5.78
Syrup of orange flowers	32	3.81 ± 0.21	5.53 ± 0.26	1.72 ± 0.14	5.20
Syrup of citric acid	31	4.10 ± 0.27	5.81 ± 0.22	1.71 ± 0.24	4.94
Syrup of tolu balsam	32	3.87 ± 0.24	5.56 ± 0.27	1.69 ± 0.17	4.76
Syrup of acacia	32	3.84 ± 0.24	5.44 ± 0.25	1.60 ± 0.13	4.60
Syrup of orange	32	3.72 ± 0.20	5.31 ± 0.24	1.59 ± 0.17	5.13
Aromatic syrup of eriodictyon	31	3.84 ± 0.19	5.39 ± 0.20	1.55 ± 0.15	5.60
Syrup of cherry	32	4.06 ± 0.23	5.59 ± 0.22	1.53 ± 0.17	4.78
Syrup of cocoa, N. F. V	32	4.00 ± 0.23	5.50 ± 0.26	1.50 ± 0.16	4.35
Syrup of prepared cacao, N. F. VI	32	3.97 ± 0.21	5.44 ± 0.27	1.47 ± 0.16	4.29
Syrup of thyme	30	3.73 ± 0.18	5.16 ± 0.25	1.43 ± 0.18	4.60
Syrup of wild cherry	32	3.78 ± 0.23	5.19 ± 0.22	1.41 ± 0.11	4.39
Syrup of cinnamon	32	4.03 ± 0.25	5.41 ± 0.25	1.38 ± 0.17	3.87
Syrup of althea	32	3.81 ± 0.23	5.09 ± 0.24	1.28 ± 0.16	3.89
Elixir of glycyrrhiza	32	3.75 ± 0.21	5.03 ± 0.26	1.28 ± 0.19	3.80
Compound syrup of sarsaparilla	32	4.03 ± 0.18	5.13 ± 0.20	1.10 ± 0.13	4.01
Aromatic elixir	32	3.78 ± 0.21	4.81 ± 0.24	1.03 ± 0.11	3.21
Syrup (simple)	32	4.15 ± 0.26	5.15 ± 0.23	1.00 ± 0.17	2.92

[a] Each vehicular solution contained 10% by volume of the respective vehicle; the remainder of the solvent was distilled water.

[b] Calculated from $\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$, the significance of differences between the thresholds for distilled water and for each of the vehicles is given in this column. Since each value is greater than 1.96, there is a significant difference between the aqueous and vehicular thresholds.

Vol. N: 31 (1942)

TABLE II.—SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN AMMONIUM CHLORIDE DISGUISING POTENTIALS FOR THE VEHICLES GIVEN IN TABLE I

Vehicles arranged in order of decreasing mean disguising potentials. Values of 1.96 or greater indicate significant difference. To find the value for any pair of vehicles, read down the vertical column beneath the name of the first to the junction of the horizontal column at the left of the second.

3.94	4.28	3.91	2.91	3.09	2.59	2.87	2.46	2.40	2.32	2.17	2.16	1.96	2.05	1.61	1.34	1.61	1.12	Syrup of Glycyrrhiza
3.03	3.35	2.95	1.93	2.07	1.64	1.74	1.41	1.31	1.21	1.06	1.02	0.84	0.87	0.45	0.32	0.37		Syrup of Raspberry
3.26	3.88	3.30	1.91	2.12	1.58	1.78	1.30	1.18	1.07	0.88	0.83	0.60	0.63	0.14	0.04			Syrup of Orange Flowers
2.41	2.59	2.26	1.42	1.51	1.14	1.15	0.95	0.84	0.74	0.62	0.57	0.41	0.40	0.07				Syrup of Citric Acid
2.84	3.22	2.76	1.63	1.77	1.30	1.39	1.06	0.94	0.82	0.67	0.61	0.42	0.41					Syrup of Tolu Balsam
2.74	3.24	2.69	1.40	1.55	1.03	1.10	0.77	0.62	0.49	0.33	0.25	0.05						Syrup of Acacia
2.45	2.77	2.31	1.24	1.35	0.89	0.91	0.66	0.52	0.40	0.25	0.18							Syrup of Orange
2.38	2.72	2.25	1.12	1.23	0.75	0.75	0.51	0.36	0.23	0.09								Aromatic Syrup of Eriodictyon
2.20	2.46	2.03	1.00	1.09	0.64	0.60	0.41	0.26	0.13									Syrup of Cherry
2.15	2.44	1.98	0.91	1.00	0.53	0.48	0.30	0.13										Syrup of Cocoa, N. F. V
1.98	2.22	1.79	0.77	0.84	0.39	0.31	0.17											Syrup of Prepared Cacao, N. F. VI
1.73	1.90	1.50	0.58	0.63	0.21	0.10												Syrup of Thyme
2.00	2.42	1.83	0.61	0.68	0.15													Syrup of Wild Cherry
1.58	1.74	1.33	0.40	0.44														Syrup of Cinnamon
1.20	1.29	0.89	0															Syrup of Althea
1.10	1.15	0.79																Elixir of Glycyrrhiza
0.46	0.41																	Compound Syrup of Sarsaparilla
0.14																		Aromatic Elixir
																		Syrup (Simple)

only to inherent variations in the subjects, calculations employing the formula $\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$ were made. The terms m_1 and m_2 represent the two means and ϵ_1 and ϵ_2 are their respective standard errors. Most workers agree that if the value calculated from the formula is 1.96 or greater, then the two results may be regarded as differing significantly. That the 19 vehicles employed actually possess the property of disguising the salty taste of ammonium chloride in comparison with an aqueous solution of the salt can be seen from the last column in Table I. Every value in this column is greater than 1.96, which means that the threshold for the diluted vehicle in every case is significantly greater than that for distilled water.

Next, the formula $\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$ was applied to the mean disguising potentials and their standard errors to find out which vehicles are definitely better disguising agents than others. The values so derived are presented in Table II. An examination of these values leads to the following observations. Neither of two vehicles lying adjacent in the table can be claimed to be superior to the other as a disguising agent for ammonium chloride. Syrup of glycyrrhiza is not significantly better as a masking agent than the syrups of raspberry, orange flowers, citric acid and tolu balsam. Syrup of glycyrrhiza is, however, a definitely better disguising agent than any of the vehicles falling below syrup of tolu balsam. The values derived from the comparison of the results for the six vehicles appearing at the bottom of the table reveal that none of these preparations differ significantly from the others in disguising properties. The vehicles from syrup of glycyrrhiza down to syrup of tolu balsam, inclusive, are the best of the vehicles tested for disguising the taste of ammonium chloride; those from simple syrup up to and including syrup of cinnamon are the poorest; and those lying between syrup of tolu balsam and syrup of cinnamon are mediocre in disguising properties. The disguising potentialities for ammonium chloride of any pair of vehicles, the comparative value for which is not enclosed in heavy lines, are not significantly different. Any two vehicles, the value for which is enclosed by heavy lines, are different in their disguising efficacy. The practical value of this lies in the fact that the physician can choose from the vehicles possessing the greatest masking properties. In the event that one of the upper five vehicles may not be palatable to a particular patient for whom a physician is prescribing, he may select one of the other four.

(*B*) *Comparison of Efficacy of 19 Official Vehicles in Disguising the Bitterness of Quinine.*—The procedure used for the evaluation of the relative salt disguising properties of 19 official syrups and elixirs was repeated, using quinine hydrochloride in place of ammonium chloride. Naturally, bitterness rather than saltiness was the end point sought in these determinations.

This work was carried out at a time when it was known in advance that the attendance of a number of the subjects would be rather irregular. For this reason, a group of 37 persons was chosen to serve as tasters in order to have a minimum of 30 for any one determination.

TABLE III.—QUININE HYDROCHLORIDE DISGUISING EFFICACY OF 19 DILUTED VEHICLES[a]

Vehicle	Number of Subjects Tested	Mean Threshold in Water ± Standard Error, $m_2 \pm \epsilon_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm \epsilon_1$	Mean Disguising Potential ± Standard Error, $D \pm \epsilon$	Significance of Difference[b]
Syrup of prepared cacao, N. F. VI	30	3.50 ± 0.31	8.17 ± 0.40	4.67 ± 0.26	9.18
Syrup of glycyrrhiza	32	3.47 ± 0.27	7.88 ± 0.59	4.41 ± 0.49	6.80
Syrup of cocoa, N. F. V	30	3.87 ± 0.26	8.27 ± 0.40	4.40 ± 0.29	9.24
Aromatic syrup of eriodictyon	32	3.84 ± 0.27	7.78 ± 0.50	3.94 ± 0.38	6.94
Syrup of thyme	30	3.67 ± 0.23	7.40 ± 0.27	3.73 ± 0.25	10.60
Elixir of glycyrrhiza	30	3.73 ± 0.25	6.93 ± 0.42	3.20 ± 0.34	6.52
Syrup of tolu balsam	30	3.47 ± 0.27	6.50 ± 0.31	3.03 ± 0.21	7.37
Compound syrup of sarsaparilla	30	3.53 ± 0.28	6.53 ± 0.38	3.00 ± 0.23	6.34
Syrup of raspberry	33	3.61 ± 0.24	6.12 ± 0.32	2.51 ± 0.27	6.20
Syrup of cherry	33	3.61 ± 0.29	6.09 ± 0.40	2.48 ± 0.27	4.99
Syrup of cinnamon	31	3.74 ± 0.21	6.13 ± 0.33	2.39 ± 0.23	6.08
Syrup of citric acid	30	3.80 ± 0.25	6.13 ± 0.36	2.33 ± 0.27	5.33
Syrup of althea	31	3.55 ± 0.27	5.77 ± 0.34	2.22 ± 0.19	5.12
Syrup of wild cherry	30	3.63 ± 0.23	5.80 ± 0.41	2.17 ± 0.33	4.59
Syrup (simple)	31	3.48 ± 0.25	5.64 ± 0.31	2.16 ± 0.19	5.44
Syrup of acacia	33	3.58 ± 0.23	5.70 ± 0.30	2.12 ± 0.22	5.61
Syrup of wild cherry	36	3.44 ± 0.25	5.47 ± 0.41	2.03 ± 0.29	4.19
Syrup of orange flowers	31	3.45 ± 0.27	5.48 ± 0.31	2.03 ± 0.18	4.94
Syrup (simple)	31	3.97 ± 0.26	5.87 ± 0.40	1.90 ± 0.23	3.97
Syrup of orange	31	3.45 ± 0.27	5.19 ± 0.40	1.74 ± 0.26	3.59
Aromatic elixir	32	3.38 ± 0.25	4.97 ± 0.32	1.59 ± 0.17	3.88

[a] Each vehicular solution contained 10% by volume of the respective vehicle; the remainder of the solvent was distilled water.

[b] Calculated from $\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$, the significance of differences between the thresholds for distilled water and for each of the vehicles is given in this column. Since each value is greater than 1.96, there is a significant difference between the aqueous and vehicular thresholds.

TABLE IV.—SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEAN QUININE HYDROCHLORIDE DISGUISING POTENTIALS FOR THE VEHICLES GIVEN IN TABLE III

Vehicles arranged in order of decreasing mean disguising potentials. Values of 1.96 or greater indicate significant difference. To find the value for any pair of vehicles, read down the vertical column beneath the name of the first to the junction of the horizontal column at the left of the second.

9.87	8.01	7.94	8.41	6.80	7.54	7.87	5.92	7.63	6.27	6.57	5.82	5.82	4.81	4.96	3.40	2.59	1.58	0.70	0.47		Syrup of Prepared Cacao, N. F. VI
5.39	4.79	4.60	4.54	4.16	4.25	4.27	3.77	4.14	3.70	3.71	3.43	3.39	2.59	2.58	2.01	1.23	0.75	0.02			Syrup of Glycyrrhiza
8.41	6.91	6.78	7.05	5.84	6.37	6.57	5.09	6.36	5.28	5.48	4.87	4.85	3.81	3.89	2.68	1.75	0.96				Syrup of Cocoa, N. F. V
5.60	4.76	4.54	4.53	3.98	4.14	4.18	3.49	4.02	3.44	3.47	3.11	3.07	2.10	2.09	1.44	0.46					Aromatic Syrup of Eriodictyon
6.99	5.51	5.32	5.52	4.43	4.84	5.00	3.73	4.78	3.79	3.92	3.36	3.32	2.14	2.15	1.24						Syrup of Thyme
4.19	3.40	3.13	3.03	2.61	2.66	2.66	2.16	2.50	2.00	1.96	1.64	1.59	0.48	0.42							Elixir of Glycyrrhiza
5.35	3.91	3.63	3.69	2.83	3.04	3.13	2.19	2.89	2.07	2.07	1.61	1.55	0.10								Syrup of Tolu Balsam
4.91	3.65	3.35	3.35	2.63	2.79	2.84	2.05	2.62	1.90	1.88	1.46	1.40									Compound Syrup of Sarsaparilla
2.91	2.08	1.73	1.51	1.22	1.14	1.08	0.80	0.89	0.48	0.34	0.08										Syrup of Raspberry
2.76	1.97	1.62	1.39	1.14	1.04	0.97	0.72	0.79	0.39	0.25											Syrup of Cherry
2.78	1.88	1.49	1.24	0.98	0.85	0.78	0.55	0.57	0.17												Syrup of Cinnamon
2.32	1.59	1.21	0.94	0.76	0.61	0.52	0.38	0.34													Syrup of Citric Acid
2.46	1.50	1.06	0.73	0.55	0.35	0.23	0.13														Syrup of Althea
1.55	1.02	0.67	0.37	0.32	0.13	0.03															Syrup of Wild Cherry
2.24	1.32	0.87	0.51	0.38	0.14																Syrup (Simple)
1.91	1.13	0.69	0.33	0.25																	Syrup of Acacia
1.31	0.75	0.35	0																		Syrup of Wild Cherry
1.78	0.93	0.45																			Syrup of Orange Flowers
1.07	0.46																				Syrup (Simple)
0.48																					Syrup of Orange
																					Aromatic Elixir

The strengths of the solutions of quinine hydrochloride used ranged from 0.0000029 *N* to 0.00056 *N* and the concentrations of consecutively numbered solutions varied as the ratio 1 : 1.5. All of the strengths employed and the numbers used to designate these strengths were as follows:

Normality	Number	Normality	Number
0.0000029	1	0.000050	8
0.0000044	2	0.000075	9
0.0000067	3	0.00011	10
0.000010	4	0.00017	11
0.000015	5	0.00025	12
0.000022	6	0.00038	13
0.000033	7	0.00056	14

The results of these determinations are presented in Table III in the order of decreasing mean disguising potentials.

In order to determine the significance of the differences between the mean disguising potentials given in Table III, the formula $\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$ was applied and the values appearing in Table IV were obtained.

It will be observed that simple syrup and syrup of wild cherry each appear twice in Tables III and IV. Threshold determinations employing these syrups were made on the first and second working days during this series of determinations, and then they were repeated after determinations in all of the other vehicles had been made. The amount of time which elapsed between the two determinations in either of the two syrups was 41 days. This repetition was to determine the effect, if any, of experience in tasting on disguising potentials. Prior to this work, none of the subjects had had any experience in making bitter threshold determinations. It was found that the two mean disguising potentials for simple syrup appearing in Table III differ by approximately 12% and that those for syrup of wild cherry differ by about 6%. These differences are not significant, as can be seen by inspection of the values appearing in Table IV. From this, it is concluded that satisfactory results can be obtained regardless of whether the subjects have had previous experience in the determination of taste thresholds.

A study of Table IV discloses that N. F. VI syrup of prepared cacao, syrup of glycyrrhiza, N. F. V syrup of cocoa and aromatic syrup of eriodictyon are the best of the 19 vehicles for masking the bitter taste of quinine hydrochloride. Other conclusions drawn from this table would be analogous to those in the discussion of Table II.

Now that the relative value of various pharmaceutical vehicles as masking agents for ammonium chloride and quinine hydrochloride has been determined, a question arises. Will the vehicles which are effective in disguising the tastes of these substances be effective to the same degree in disguising the salty and bitter tastes of other substances? It is very likely that the answer would be in the affirmative. However, before any definite answer can be given, it will be necessary to experiment with other salty and bitter drugs.

The procedure described herein should be equally satisfactory for similar determinations in any vehicle, using drugs possessing tastes other than salty or bitter.

CONCLUSIONS

1. A satisfactory method for the comparison of the taste-disguising properties of pharmaceutical vehicles has been devised. This method is dependent upon the threshold of taste and the application of statistical methods for the evaluation of results.

2. The relative worth of 19 vehicles of the United States Pharmacopœia and National Formulary as agents to disguise the tastes of ammonium chloride and quinine hydrochloride has been established. Of these vehicles, the syrups of glycyrrhiza, raspberry, orange flowers, citric acid and tolu balsam afford the best disguises for ammonium chloride, whereas N. F. VI syrup of prepared cacao, syrup of glycyrrhiza, N. F. V syrup of cocoa and aromatic syrup of eriodictyon are the best to disguise the taste of quinine hydrochloride.

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The Pharmacology of Sodium Hydroxyacetate with Observations on the Toxicity of Glycine*†

By Walter F. Riker and Harry Gold

The behavior of hydroxyacetic acid (glycolic acid) in the animal organism has received some consideration in the literature (1, 2, 3, 4). Concerning the questions of whether glycolic acid is a normal product of intermediary metabolism, whether the animal organism can produce it under any conditions, and the behavior of injected material, the existing evidence is inconclusive. Observations on the effects of hydroxyacetic acid administered to animals and man are meager. Pohl (1) observed swaying of the body and drowsiness within a half-hour after the oral administration of 0.67 Gm. of sodium hydroxyacetate per Kg. in a dog. He deduced from the occurrence of these effects that it is well absorbed from the gastrointestinal tract. Milhorat and Toscani (2) did not record any symptoms in 4 patients with progressive muscular dystrophy after sodium hydroxyacetate in oral doses of the order of 0.26 to 0.4 Gm. per Kg. daily for periods up to 7 days.

The present experiments were planned to investigate further the pharmacology of hydroxyacetic acid.

EXPERIMENTAL

The experiments were performed on 35 cats, 2 dogs and 1 rabbit. The effects of the drug were studied by oral (stomach tube) and intravenous administration, after a single large dose, and after repeated oral doses for periods up to somewhat longer than 2 months.

The material was used, except when otherwise stated, in the form of a buffered mixture of hydroxyacetic acid and sodium hydroxyacetate, making a 9.8% solution with a *p*H of 7.3. It was prepared by dissolving 98 Gm. of sodium hydroxyacetate and 0.024 Gm. of hydroxyacetic acid in distilled water and diluting up to 1 L. The *p*H of the solution was checked with the Leeds-Northrup glass electrode.

DISCUSSION OF RESULTS

Some of the results on toxicity have been assembled in Table I. An oral dose of 0.1 Gm./Kg. is without effect; 0.25 Gm./Kg. usually causes some signs of poisoning; 0.5 Gm./Kg. may prove fatal; larger doses are usually fatal.

The effects resemble some of the elements of poisoning by a barbiturate, camphor, strychnine and bulbocapnine. Neuromuscular disturbances are the chief symptoms. Weakness, ataxia and anorexia are the earliest effects. The animal prefers to lie. It is easily thrown over. Its limbs sprawl. When it stands, its body sways. In the early phase of poisoning, the animal appears to be quite alert and playful, although it may be too weak to walk. The narcotic action is relatively slight. The muscular weakness progresses and the pattern of poisoning becomes complicated by signs of cerebrospinal stimulation. Catatonic disturbances appear after the larger doses. The animal assumes awkward posture with lead-pipe rigidity of the limbs. As the poisoning advances, fibrillary twitchings of the muscles appear. The gait becomes spastic. Convulsions follow at irregular intervals. At times the convulsions are camphor-like, myoclonic, without reflex hyperexcitability; at other times reflex hyperexcitability predominates and the character of the convulsions resembles more nearly those of strychnine. Convulsions are late effects; they usually developed only after many hours following the doses used and invariably terminated fatally. The pupillary reflexes remain essentially normal. The corneal reflexes are depressed in the later stages. Some analgesia is also present in more advanced poisoning. After the oral doses, signs of nausea and vomiting are very common, although they are apt to appear only after many hours. They are rare after intravenous doses. The character and severity of the symptoms depend on the doses. They are essentially similar after oral and intravenous administration.

The effects described above were substantially the same in the dog. An oral dose of 1.5 Gm./Kg. resulted in weakness, ataxia, nausea and vomiting. These effects progressed and were fairly marked 24 hrs. later, when they were greatly intensified by an intravenous injection of 1 Gm./Kg. The weakness progressed further during the next 5 days when myoclonic convulsions appeared, resulting in death on the sixth day. In another dog an intravenous dose of 1.5 Gm./Kg. caused similar symptoms but without convulsions and with recovery in about 24 hrs. There remains some question about its effect in the rabbit. An intravenous injection of 1 Gm./Kg. produced no perceptible effects on a rabbit. This requires further study.

Absorption.—The absorption of sodium hydroxyacetate from the gastrointestinal tract is fairly complete as judged by the fact that the oral and intravenous fatal doses are not far apart. It may be

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TABLE I.—TOXICITY OF SODIUM HYDROXYACETATE

Dose, Gm./Kg. Single	Dose, Gm./Kg. Total	Route	Severity of Effects (Symptoms)	Remarks
			Single Dose	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.10		Oral	0	
0.25		Oral	0	
0.25		Oral	1+	Recovered in 2 days
0.25		Oral	1+	Recovered in 3 days
0.25		Oral	1+	Died with pneumonia
0.25		Oral	1+	Died after repeated doses in 21 days
0.50		Oral	2+	Died with pneumonia in 16 days
0.50		Oral	4+	Died in 5 days
0.50		Oral	2+	Died after repeated doses in 9 days
1.00		Oral	2+	Recovered in 1 day
1.50		Oral	4+	Died in 6 days
2.00		Oral	4+	Died in 1½ days
2.50		Oral	5+	Died in 1½ days
1.00		Vein	1+	Recovered in 1 day
1.00		Vein	2+	Recovered in 4 days
1.00		Vein	4+	Died in 4 days
1.00		Vein	4+	Died in 2 days
1.27		Vein	4+	Died in 3 days
1.50		Vein	4+	Died in 2 days
1.50		Vein	3+	Died after intravenous calcium
1.50		Vein	3+	Died after intravenous calcium
2.00		Vein	3+	Died in 4 days
2.40		Vein	4+	Died in 1 day
			Repeated Doses	
0.10	2.30	Oral	0	
0.10	2.30	Oral	0	
0.10	2.30	Oral	0	
0.10	3.00	Oral	0	
0.10	3.00	Oral	0	
0.25	2.25	Oral	4+	Died in 21 days
0.25	3.25	Oral	0	Marked azotemia without symptoms; dose discontinued, partial recovery
0.50	3.00	Oral	4+	Died in 9 days

seen in Table I that 2 of 4 animals survived 1 Gm./Kg. by intravenous injection, and that as little as 0.5 Gm./Kg. by oral administration may prove fatal. The absorption, however, is quite slow, as indicated by a comparison of the speed of onset of effects after oral and after intravenous administration. For example, a dose of 1 Gm. by intravenous injection produced the first effects within 26 min.; by oral administration, within 78 min. The intravenous doses produced effects as a rule within less than 30 min., whereas even fatal oral doses rarely showed effects in less than 1 hr.

Speed and Duration of Action.—There is a long latent period in the development of the effects of sodium hydroxyacetate. This is not a matter of absorption, since it also applies to intravenous doses. Its duration depends on the dose; after a small oral dose of 0.25 Gm./Kg., as long as 18 hrs. may elapse before distinct symptoms of poisoning are in evidence; with the larger intravenous doses (1 Gm./Kg. or more), effects may be in evidence as early as 10 min. after administration, although usually characteristic signs of poisoning are delayed for as long as one-half hour to an hour. The course of the poisoning is protracted, and, as seen in Table I, terminates in death or recovery after periods of days. It may require as long as 2 to 3 days for manifest signs of poisoning to subside after a dose as small as 0.25 Gm./Kg. In cases which prove fatal, the period of poisoning may be longer, up to 21 days.

Cumulation.—A cat which received an oral dose of 0.5 Gm./Kg. in equal daily fractions during a 5-day period remained without signs of poisoning, while another cat which received a similar dose at one time developed poisoning by the following day which progressed and proved fatal at the end of 5 days. The effect of repeated doses was studied in a group of 8 animals. An oral dose was given daily during the first week and then every second day. The results of those experiments in which the blood chemistry was followed are summarized in Table II.

TABLE II.—EFFECT OF SODIUM HYDROXYACETATE ON BLOOD AND URINE IN THE CAT

No. of Cats	Dose: Single, Gm./Kg.	Dose: Total No.	Total Dose, Gm./Kg.	Experimental Day	Blood: Hb., Gm. %	Blood: R. B. C., Million per Cu. Mm.	Blood: W. B. C., per Cu. Mm.	Blood: N. P. N., Mg. %	Blood: Creatinine, Mg. %	Blood: Prothrombin, %	Urine: Sp. Gr.	Urine: Albumin and Sugar
6	Control	..	....	2	....	....	....	32	1.8	...	....	0
				8	....	....	....	35	1.4	...	1.058	0
				13	....	....	....	35	1.4	...	1.058	0
				33	....	....	....	34	1.1	...	1.042	0
				47 to 48	14.5	7.5	9,400	46	1.7	...	1.054	0
				54 to 56	11.0	7.5	6,200	...	....	100	....	...
				68	....	....	....	38	1.4	93	1.050	0
				82	....	....	....	39	1.6	87	1.070	0
				91	12.5	8.3	8,300	...	....	...	....	...
				96	....	....	....	35	1.4	94	1.050	0
				110	....	....	....	40	1.4	83	1.050	0
				120	13.0	7.8	5,500	...	....	...	....	...
				126	....	....	....	44	1.3	92	....	...
1	0.1	30	0	−10 to −8	16.0	11.7	10,400	54	1.8	100	1.032	0
			0	− 3 to −1	15.5	7.9	8,000	44	0.9	83	Q. N. S.	0
			0.6	11	....	....	....	31	1.7	100	1.054	0
			0.8	16	11.0	9.2	7,800	...	....	...	....	...
			1.1	25	....	....	....	39	1.4	75	1.050	0
			1.5	34	9.0	6.0	13,100	...	....	...	....	...
			1.7	39	....	....	....	41	1.1	100	1.048	0
			2.4	53	....	....	....	49	1.5	100	1.052	0
			2.8	63	10.0	6.0	11,200	...	....	...	....	...
			3.0	69	....	....	....	46	1.3	85	....	...
11	0.25	9	0	−10 to −8	12.0	7.8	10,600	55	1.5	...	1.060	0
			0	− 3 to −1	9.0	7.0	5,200	43	1.1	83	....	0
			1.5	11	....	....	....	106	2.5	98	1.030	0
			2.0	16	12.0	7.5	17,000	...	....	...	....	...
			2.25	19	....	....	....	197	4.2	...	....	...
10	0.50	6	0	−10 to −8	10.0	6.5	8,300	52	1.4	...	1.040	0
			0	− 3 to −1	9.0	6.8	9,100	53	1.3	78	....	0
			3.0	8	....	....	....	328	11.7	79	....	...

In the 2 cats in which the daily dose was 0.1 Gm./Kg., no effects were in evidence after 30 doses (total dose, 3 Gm./Kg. in a period of 69 days), indicating elimination of approximately 43 mg. of the agent per Kg. per day. Another cat which received a repeated oral dose of two and one-half times as much, or 0.25 Gm./Kg., developed signs of poisoning with azotemia and died in 21 days, after a total of 2.25 Gm. If the rate of elimination is a straight line function of the dose, the amount remaining after elimination during the 21 days would not be sufficient to cause poisoning in this case. The fact that the animal died indicates that some factor may have interfered with elimination. While there are other possible explanations, the indications are that the nephrotoxic action of this compound interferes with its excretion, and may explain the observation that whereas nontoxic doses when repeated show no tendency to cumulation (5 animals received 0.1 Gm./Kg. daily in Table I), recovery is slow and cumulation is marked after doses which cause toxic effects.

Heart, Circulation and Respiration.—The effects were observed in 6 cats. The mean blood pressure was recorded from the carotid artery with a mercury manometer on a smoked drum, the respiration from a pleural cannula. Electrocardiographic tracings were taken for the effect on the heart.

Three cats under dial anesthesia received intravenous injections of hydroxyacetic acid as the acid in a 5% solution (*p*H 3.4). These produced marked hyperpnea with fall of the blood pressure and death with circulatory collapse and depression of respiration after total doses of 178, 225 and 312 mg. per Kg., respectively. Undoubtedly, nonspecific acid effects were obtained. The buffered mixture was used in the subsequent experiments. One cat received an intravenous injection of 1.35 Gm./Kg. in the form of a 5% solution. This produced no effect on the respiration or the blood pressure within a period of 15 min. In another cat the experiment was performed during local anesthesia with procaine. A dose of 0.4 Gm./Kg. was administered at intervals of 5 to 14 min., a total of 2.4 Gm. in a period of 55 min. There was no effect on the respiration. The blood pressure declined from an initial level of 190 mm. of mercury to 150 mm., while the heart rate accelerated somewhat. The following day the animal was moribund, and at this time the heart rate had fallen to 40/min. In the case of another cat, a blood pressure record was taken during the period of advanced symptoms of poisoning with marked azotemia. This animal had received repeated oral doses of 0.25 Gm./Kg. during 21 days (total dose, 2.25 Gm./Kg.). At the end of this period the N. P. N. was 197 mg. % and creatinine, 4.2 mg. %, and the mean blood pressure was 135 mm. of mercury.

It is clear from these experiments that when pH disturbances are excluded, hydroxyacetic acid exerts relatively insignificant direct effects on the blood pressure and respiration. Even during severe poisoning, the blood pressure, though lower than the average blood pressure for a normal cat, was fairly well sustained.

Electrocardiograms were taken at intervals in 4 cats which received repeated oral doses of 0.1 and 0.25 Gm. per Kg. No significant changes in the tracings were observed even during the stage of severe toxic symptoms.

Changes in Urine and Blood Chemistry.—The urine was examined for albumin (heat and acetic acid) and for sugar (Benedict's solution). The blood was examined for N. P. N. (Folin and Denis (5)), creatinine (Folin and Wu (6)), and prothrombin (Warner, Brinkhous and Smith (7)). Typical results are summarized in Table II.

Three cats were studied as controls of the effect of the laboratory conditions and diet on the blood and the urine for 4 months. They remained normal in every respect. A constant diet of dog food ration, chopped meat and fresh liver was used in the control as well as the treated animals.

Each of 5 cats received daily oral doses of sodium hydroxyacetate varying from 0.1 to 0.5 Gm. per Kg. As previously described, the larger doses caused symptoms of poisoning and death. The urine remained negative for albumin and sugar. The specific gravity of the urine remained essentially unchanged. This was also true in those cases in which signs of renal insufficiency developed as shown by the azotemia. There were no consistent changes in the number of red blood cells, white blood cells, or the amount of hemoglobin. The prothrombin time remained unchanged. Doses of 0.1 Gm./Kg. repeated daily caused no change in the N. P. N. or creatinine. The larger doses, 0.25 Gm. and 0.5 Gm. per Kg. repeated daily, however, caused marked elevation of the N. P. N. and creatinine. In one animal it was observed that this change is partially reversible; the N. P. N. rose from 41 to 123 mg. %, and the creatinine from 1.5 to 2.4 mg. % during the use of the 0.25 Gm./Kg. dose. The drug was then discontinued, and both the N. P. N. and creatinine fell off, the creatinine to the normal level, the N. P. N. showing only partial recovery, namely, 67 mg. %. In this animal sodium hydroxyacetate produced a marked nephrotoxic action with azotemia, although the appearance and behavior of the animal remained normal during the period of observation.

The indications are that sodium hydroxyacetate is less nephrotoxic than tartrate, although we do not have sufficient data for a precise comparison. Previously (8), it was found that 0.3 Gm. of tartrate per Kg. caused severe renal damage. In a cat of the present study, 0.25 Gm. of sodium hydroxyacetate per Kg. repeated at intervals of 24 to 48 hrs. during a period of 11 days (total dose, 1.5 Gm./Kg.) produced no azotemia, although the continued use of this dose resulted in marked renal damage with azotemia.

It should be noted, however, that the renal damage is not the cause of most of the early toxic symptoms, since an animal may show severe poisoning within an hour or two after the dose, while the cat with bilateral nephrectomy goes without serious effects for more than 24 hrs. Furthermore, after sodium hydroxyacetate, the cat may develop severe tubular nephritis without general signs of poisoning.

Pathological Changes.—Gross autopsy findings in the case of animals that died from the drug or were sacrificed after doses of the drug which did not prove fatal were, in the main, negative. No significant changes were observed in the heart, lungs, liver, kidneys, spleen, adrenals or gastrointestinal tract. After the large intravenous doses, small pulmonary hemorrhages were seen, but these are probably nonspecific, since large intravenous injections of sodium chloride also produce them. The brain was examined in gross sections in 2 cases, and the findings were also negative. Microscopic sections of the kidney and liver were made in the case of 5 animals which received daily oral doses as follows: 0.1, 0.25 and 0.5 Gm. per Kg. (total dose, 2.25, 3.0 and 3.25 Gm. per Kg.). The liver showed no changes. The kidneys showed tubular degeneration in all but one case (0.1 Gm./Kg.). It was slight in 1 cat which received the dose of 0.1 Gm./Kg. In this cat the blood chemistry remained normal. The tubular degeneration was more pronounced after the larger doses, and corresponded to the azotemia. The histological changes in the kidneys are similar to those observed after the tartrates.

Mechanism of Action.—The anorexia, and the neuromuscular disturbances with weakness and convulsive symptoms, suggested several possible mechanisms of action: a curare-like action, acidosis, disturbed calcium metabolism (since the possibility of oxalate formation from hydroxyacetic acid has been suggested), disturbance in sugar metabolism, interference with metabolism of thiamine. These possibilities were tested chiefly by attempts to counteract the toxic effects of sodium hydroxyacetate. The measures used failed to influence them, and hence indicate that the foregoing factors are probably not involved.

A cat received an oral dose of 1 Gm./Kg.; and when the poisoning was pronounced, 0.24 mg. of physostigmine salicylate per Kg. was injected intramuscularly. There was no effect on the neuromuscular weakness. In 2 cats the phrenic and femoral nerves were stimulated with the Harvard inductorium during severe poisoning with intravenous doses of 1.3 and 5 Gm. per Kg., respectively. The response of the diaphragm and the leg muscles appeared to be normal. In 2 additional cats, the tension developed by the gastrocnemius muscle was measured by means of an isometric lever with the technique described by Wolff and Cattell (9), after intravenous doses of 1 and 1.6 Gm. per Kg., respectively. The drug produced no change in tension developed by the muscle during contraction. These types of experiments exclude a curare-like action.

TABLE III.—TOXICITY OF SEVERAL ORGANIC ACIDS

No. of Cats	Drug	Dose	Systemic Poisoning	Remarks
1	Sodium fumarate	5 Gm./Kg., oral, 15% soln.	None during 6 days	Vomiting and diarrhea, vomitus returned
1	Sodium citrate	2 Gm./Kg., oral, 20% soln.	None during 9 days	Repeated vomiting, about 1/2 of dose lost
1	Sodium citrate	1 Gm./Kg., oral, 5% soln.	None during 5 days	
4	Sodium acetate in buffered mixture with acetic acid	1 to 2.5 Gm./Kg., oral, 20% soln.	None	
1	Sodium acetate in buffered mixture with acetic acid	1 Gm./Kg., vein, 20% soln.	None	
1	Glycine[a]	3 Gm./Kg., oral, 20% soln.	None in 50 min.	After 50 min., next dose given
		2.5 Gm./Kg., oral, 20% soln.	None during 4 days	Vomited in 75 min.
1	Glycine	6 Gm./Kg., oral, 20% soln.	Profuse drooling for 3 hrs., otherwise none during 5 days	Vomited most of dose in 10 min.
1	Glycine	6 Gm./Kg., oral, 10% soln.	Weakness, swaying, awkward posture in 25 min.; convulsions in 75 min.; pupils dilated and do not react to light	Partial recovery over 5-day period
1	Glycine	1 Gm./Kg., vein, 10% soln.	Drooling, muscular weakness and reflex hyperexcitability in 25 min.; pupils dilated and do not react to light	Recovery complete in 4 hrs.
1	Glycine	2 Gm./Kg., vein, 10% soln.	Same as after 1 Gm., but more marked; also vomiting	Recovery complete in 24 hrs.
2	Glycine	3 Gm./Kg., vein, 10% soln.	Same as after 2 Gm., but more marked; convulsions	One died in 1 hr., 45 min.; the other in 10 hrs.

[a] Effects the same when given as the acid as when neutralized with sodium bicarbonate.

In one cat which received an intravenous dose of 1 Gm./Kg., a control sample of blood taken from the jugular vein showed a carbon dioxide combining power of 44 vols. % (manometric method of Van Slyke) and sugar, 135 mg. %. A sample taken 2 hrs. after the dose, when symptoms of poisoning were pronounced, showed a carbon dioxide combining power of 47, and blood sugar, 143. The effects of sodium hydroxyacetate, therefore, appear not to be related to acidosis or disturbance in blood sugar level.

In one cat which received an intravenous dose of 1.5 Gm./Kg., repeated intravenous injections of a 10% solution of calcium chloride (total, 160 mg./Kg.) had no effect on the neuromuscular disturbance. In this animal an intravenous injection of 0.25 mg. of thiamine hydrochloride per Kg. was also without effect on the muscular weakness.

In one cat which received an intravenous injection of 1 Gm./Kg., and developed marked symptoms of poisoning, the intravenous injection of a 10% solution of glucose in a total dose of 3.7 Gm./Kg. was without influence on the course of the symptoms.

While, therefore, certain possibilities regarding the mechanism of the toxic effect of sodium hydroxyacetate have been excluded, the mechanism of this action remains uncertain.

Comparison with Some Other Organic Acids.—The toxicity of sodium hydroxyacetate was compared with acetate, fumarate and citrate in a series of cats, the results of which are presented in Table III. The latter three compounds produced no systemic toxic effects in doses varying from 4 to 20 times the toxic dose of sodium hydroxyacetate.

Aminoacetic acid (glycine) presents a problem of special interest in relation to the toxicity of sodium hydroxyacetate, since it has been suggested that glycine may be a precursor of hydroxyacetic acid in the synthesis of creatine (Davenport, *et al.* (3)), or that hydroxyacetic acid is an intermediary in the conversion of glycine into glucose (Lusk (4), or, inversely, that hydroxyacetic acid may be a precursor of glycine (Milhorat and Toscani (2)). The toxicity of glycine has received little attention in the literature. Ni (10) found glycine toxic to guinea pigs in oral doses of the order of 2 Gm./Kg., with symptoms of muscular weakness and nervous disturbances. Lewis (11), who injected glycine intravenously in dogs in a study of the rate of metabolism of glycine, recorded the incidental observation that one dog developed vomiting, diarrhea and convulsions, after what appears to have been a total of about 1.5 Gm./Kg. He stated that these symptoms disappeared when the injection was stopped.

In the present study 7 cats received glycine orally and intravenously in doses ranging from 1 to 6 Gm. per Kg. The results are also summarized in Table III. Glycine, in doses larger than those commonly used, is toxic both by oral and intravenous administration. The indications are that it possesses about one-sixth to one-third of the toxicity of

sodium hydroxyacetate. About 3 Gm./Kg. by intravenous injection may prove fatal, and about 6 Gm./Kg. by oral administration may be survived. The symptoms of glycine poisoning are in some respects similar to, but not identical with those of sodium hydroxyacetate. Both cause nausea and vomiting, muscular weakness, ataxia and convulsions. Glycine, however, produces profuse drooling and a pupillary change (complete dilatation of the pupil which may fail to react to light) which we have not seen with sodium hydroxyacetate. In one dog an oral dose of 6 Gm./Kg. caused similar symptoms with convulsions and death in 4 hrs.

Metabolism of Hydroxyacetic Acid.—It has been shown in several studies (1, 2) that the conversion of hydroxyacetic acid into oxalic acid which takes place *in vitro* does not occur in animals or man. In several of the cats of the present study, specimens of urine reduced Benedict's solution after sodium hydroxyacetate. Since the suggestion has been made that hydroxyacetic acid might be a source of glucose (Lusk (4)), blood sugar determinations were made over a period of 5 to 7 hrs. in a group of 6 cats after intravenous doses of 1 Gm./Kg. No elevation of the blood sugar resulted. The urine in these animals also frequently showed a positive reaction with sodium nitroprusside. The possibility of the conversion of sodium hydroxyacetate into acetone bodies was considered. In 4 of the foregoing 6 animals, total acetone bodies in the blood were determined by the salicylaldehyde method of Behre (12). The total acetone bodies was not elevated.

A comparison of the toxic effects of glycine and sodium hydroxyacetate throws some light on the question of conversion of one into the other. If the conversion were quantitative and took place fairly rapidly, a gram of glycine should produce the toxic effects of approximately a gram of sodium hydroxyacetate. However, the present experiments show that sodium hydroxyacetate is about 3 to 6 times as toxic as glycine. Furthermore, the character of the symptoms in glycine poisoning are in some respects different. The evidence obtained in the present study indicates, therefore, that the conversion of glycine into hydroxyacetic acid, if it occurs at all, takes place to a very small extent.

Toxicity of Sodium Hydroxyacetate in Man.—We have made no experiments in humans bearing on this matter. On the basis of body weight, the oral dose of sodium hydroxyacetate which could be repeated daily for a long period of time with little danger of poisoning in cats (0.1 Gm./Kg.) would correspond to a daily oral dose of about 7 Gm. for the average man. A dose twice as large (14 Gm. daily for a man) corresponds to a dose which caused poisoning in cats. The indications seem to be that sodium hydroxyacetate by oral administration may be more toxic for the cat than for man, since doses larger than those toxic for cats were given to 4 patients by Milhorat and Toscani (2) without apparent toxic effects. This matter requires further study.

SUMMARY AND CONCLUSIONS

1. Sodium hydroxyacetate is toxic for cats and dogs, and produces similar effects in both species.

2. An oral dose of 0.1 Gm./Kg. rarely causes toxic effects. An oral dose of 0.25 Gm./Kg. is toxic, although not fatal. An oral dose of 0.5 Gm./Kg., corresponding to about 35 Gm. for a man, may prove fatal.

3. The absorption of sodium hydroxyacetate from the gastrointestinal tract appears to be rather slow, but indications are that it is fairly complete.

4. The onset of effects of sodium hydroxyacetate is slow even after intravenous injection. The length of the latent period varies inversely with the dose. The course of action is protracted. In the typical case, effects appear after about 30 min., progress in intensity during the next 24 hrs., and either subside gradually during the subsequent several days or increase in intensity and prove fatal in several days.

5. The cat eliminates nontoxic doses of sodium hydroxyacetate within 24 hrs. or less. The recovery from toxic doses, however, is so slow as to suggest some impairment of elimination or an injury which progresses independent of the elimination of the drug.

6. The symptoms of sodium hydroxyacetate toxicity are anorexia, nausea and vomiting, neuromuscular disturbances, with weakness, ataxia, muscle twitching and convulsions.

7. Sodium hydroxyacetate exerts a nephrotoxic action resulting in tubular degeneration and marked elevation of the blood N. P. N. and creatinine.

8. Limited comparisons with other organic acids were made. It is more toxic than fumaric acid, citric acid, acetic acid and aminoacetic acid.

9. Glycine is toxic to cats and dogs, producing symptoms resembling in many respects those of sodium hydroxyacetate, but not identical with the latter.

10. Observations concerning the mechanism of action and metabolism of sodium hydroxyacetate are discussed.

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Estimation of Magnesium in Solution of Citrate of Magnesia*

By Frederick K. Bell†

Since the work of Berg (1) in 1927, the use of 8-hydroxyquinoline, or oxine, as a quantitative precipitant for certain metallic ions, has become definitely established among quantitative analytical procedures. This reagent is especially suitable for the determination of magnesium and the method offers distinct advantages over the classical phosphate procedure.

For the assay of magnesium oxide in Solution of Citrate of Magnesia the Pharmacopœia employs the phosphate method (2). It is believed that this method, even in the considerably simplified form which the Pharmacopœia prescribes, is entirely adequate in accuracy. The liberal limits for the magnesium oxide content (1.6–1.9%) are well within the therapeutic range so that the assay may be regarded to a large extent as a control of the stability of the final preparation. The present study was undertaken to determine the availability of the oxine method for this assay with the realization that the presence of relatively large amounts of sugar and citrate ion would constitute a somewhat special application of the method.

For our preliminary studies we have not attempted to prepare quantitative solutions of the official preparation. We have avoided the obvious difficulties involved in making such preparations by using a simplified solution containing the desired amount of magnesium oxide as magnesium sulfate, sugar and citric acid. Two of these simplified solutions were prepared representing the limits of magnesium oxide set by the Pharmacopœia, *i. e.*, 1.6% and 1.9%. The sugar and citric acid content of both solutions was the same and in accord with the values prescribed by the Pharmacopœia.

Two methods of procedure for the oxine precipitation were examined. In the first method (3), the slightly acid solution is treated with the required amount of oxine dissolved in acetic acid. The magnesium oxyquinolate is then precipitated by the addition of ammonia. Using this method we found it very difficult to obtain constant weight in the drying of the precipitate.

In the second method (4), the strongly ammoniacal solution, with ammonium chloride added to prevent the precipitation of the magnesium, is treated with the required amount of an alcoholic solution of the oxine. In the subsequent drying of the precipitate, constant weight could be obtained satisfactorily, and this method is therefore the one that was adopted.

For the preparation of the simplified test solutions a reagent grade of the heptahydrate of magnesium sulfate was used and was assayed in the following manner. Approximately 0.5 Gm. of the salt, accurately weighed, was dissolved in 150 cc. of distilled water previously heated to 70° to 80° C. After adding 5 cc. of a 2 *N* solution of ammonium chloride and 3 cc. of stronger ammonia, 14 cc. of a 5% solution of oxine in alcohol was added slowly with stirring. The mixture was allowed to stand for about 30 min. and then the supernatant liquid was decanted through a sintered glass filter crucible. The precipitate was washed four times by decantation with 25-cc. portions of 1% ammonia and then completely transferred to the filter crucible and washed thoroughly with distilled water. The filter crucible and contents were then dried to constant weight at 100–105° C. The results of three determinations gave values of 100.4%, 100.3% and 100.5%, respectively, corresponding to an average value of 100.4% $MgSO_4.7H_2O$.

* Received July 10, 1942, from the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, Md.

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From preliminary experiments with commercial samples of the official Solution of Citrate of Magnesia it was decided to use 2.5 cc. of the solution for the present assay. For a sample of this size the equivalent weight of the magnesium oxyquinolate varies between approximately 345 mg. for a sample containing the equivalent of 1.6% magnesium oxide and 410 mg. for a 1.9% magnesium oxide sample. Subsequent results indicate that magnesium oxyquinolate precipitates within these weight limits could be handled very rapidly and, as will be seen below, with quite satisfactory reproducibility and accuracy in the results.

As indicated above, two simplified magnesium citrate solutions were prepared. Each solution contained 9.4 Gm. of citric acid and 14.6 Gm. of sucrose per 100 cc. of solution. One solution contained an accurately weighed quantity of reagent magnesium sulfate to give approximately the equivalent of 1.6% in magnesium oxide and the other the equivalent of 1.9% magnesium oxide. The actual magnesium oxide equivalents of these solutions after proper correction for the assay of the reagent magnesium sulfate detailed above were 1.61% and 1.90%, respectively.

These two solutions were assayed according to the U. S. P. method and by the oxine method, which was carried out in the following manner. To 150 cc. of distilled water heated to 70–80° C. was added the desired amount of the test solution, followed by 1 cc. of 2 *N* ammonium chloride and 3 cc. of stronger ammonia; 8 cc. of a 5% solution of oxine in alcohol was then added slowly with thorough stirring. After standing approximately 30 min., the supernatant liquid was decanted through a weighed sintered glass filter crucible which had been previously dried at 100–105° C. The precipitate was washed three times by decantation with 20-cc. portions of distilled water and then completely transferred to the filter crucible, followed by thorough washing with distilled water. The filter and contents were dried to constant weight at 100–105° C.

Typical data of assays carried out in triplicate on these solutions are shown in Table I, using the magnesium oxide conversion factor of 0.1157 for magnesium oxyquinolate, $Mg(C_9H_6NO)_2.2H_2O$.

It is clear that the results obtained by the oxine method, as employed, are quite satisfactory both in reproducibility and desired accuracy and that therefore the presence of relatively large amounts of sugar and citric acid has no significant effect These results are consistently lower than those obtained by the U. S. P. method and agree closely with the calculated values for the magnesium oxide content.

For both the 1.61% and 1.90% solutions the same amount of oxine solution (8 cc.) was used and this amount of the reagent is sufficient to precipitate somewhat more magnesium than corresponds to 1.9% of magnesium oxide, which is the upper limit of the Pharmacopœial standard.

In applying the method to commercial samples of Solution of Citrate of Magnesia we have made one modification in the above method. After removing the excessive carbon dioxide by repeated pouring, exactly 50 cc. of the solution was pipetted into a 100-cc. volumetric flask and diluted to the mark with distilled water. Five cubic centimeters of this diluted solution, a quantity readily handled by a pipette of the conventional transfer type, was used for the assay, and 20 cc. was used for each determination by the U. S. P. method. The 5-cc. pipette and the 20-cc. pipette used throughout the study were checked with reference to the ratio of their delivery capacities, using water as the calibrating medium. This ratio was found to be very close to 1:4 and no correction was necessary for the direct comparison of the results obtained by the two methods.

Commercial samples of Solution of Citrate of Magnesia were purchased from retail druggists in a manner planned to give as wide a representation as possible of the product marketed in this community. Table II gives typical results of the assays, in triplicate, for ten of these commercial preparations.

In regard to the reproducibility of these results there is little choice between the two methods of assay. The oxine method is again found to yield results consistently lower by a few hundredths of a per cent than those obtained by the U. S. P. method. This difference in itself cannot be regarded as of great importance to the present study.

It is interesting to note that of the ten products examined, eight were labeled U. S. P. Of these, only one, sample *B*, falls below the minimum U. S. P. requirement, regardless of which of the two methods, of assay is employed. The label of sample *E* definitely states that the U. S. P. formula has not been followed and neither the label nor cap of sample *F* makes any reference whatever to the Pharmacopœia.

The final evaluation of the two methods rests upon the relative ease of manipulation and the time factor and in these respects the oxine method presents definite advantages. No ignition step is re-

TABLE I

% of MgO Calculated	U. S. P. Method			Oxine Method		
	Gm. of Pyrophosphate	Equivalent MgO in Gm.	% of MgO	Gm. of Oxyquinolate	Equivalent MgO in Gm.	% of MgO
1.61	0.4598	0.1665	1.67	0.3525	0.04078	1.63
	0.4606	0.1668	1.67	0.3514	0.04062	1.62
	0.4595	0.1663	1.66	0.3508	0.04059	1.62
1.90	0.5426	0.1965	1.97	0.4130	0.04778	1.91
	0.5389	0.1951	1.95	0.4146	0.04797	1.92
	0.5381	0.1948	1.95	0.4145	0.04796	1.92

TABLE II

Sample	U. S. P. Method			Oxine Method		
	Gm. of Pyrophosphate	Equivalent MgO in Gm.	% of MgO	Gm. of Oxyquinolate	Equivalent MgO in Gm.	% of MgO
A	0.4423	0.1602	1.60	0.3394	0.03927	1.57
	0.4454	0.1613	1.61	0.3406	0.03941	1.58
	0.4431	0.1604	1.60	0.3413	0.03949	1.58
B	0.4250	0.1539	1.54	0.3296	0.03813	1.53
	0.4272	0.1547	1.55	0.3278	0.03793	1.52
	0.4249	0.1539	1.54	0.3282	0.03797	1.52
C	0.4457	0.1614	1.61	0.3399	0.03933	1.57
	0.4432	0.1605	1.61	0.3405	0.03944	1.58
	0.4461	0.1615	1.62	0.3409	0.03944	1.58
D	0.4652	0.1684	1.68	0.3560	0.04119	1.65
	0.4651	0.1684	1.68	0.3565	0.04125	1.65
	0.4654	0.1685	1.69	0.3564	0.04124	1.65
E	0.3506	0.1270	1.27	0.2690	0.03112	1.24
	0.3493	0.1265	1.27	0.2700	0.03124	1.25
	0.3488	0.1263	1.26	0.2715	0.03141	1.26
F	0.4445	0.1610	1.61	0.3459	0.04002	1.60
	0.4457	0.1614	1.61	0.3436	0.03975	1.59
	0.4467	0.1618	1.62	0.3411	0.03947	1.58
G	0.4472	0.1619	1.62	0.3431	0.03969	1.59
	0.4464	0.1616	1.62	0.3444	0.03985	1.59
	0.4455	0.1613	1.61	0.3439	0.03979	1.59
H	0.4631	0.1677	1.68	0.3528	0.04082	1.63
	0.4645	0.1682	1.68	0.3535	0.04090	1.64
	0.4635	0.1678	1.68	0.3530	0.04084	1.63
J	0.4616	0.1671	1.67	0.3540	0.04096	1.64
	0.4600	0.1666	1.67	0.3522	0.04075	1.63
	0.4611	0.1670	1.67	0.3533	0.04088	1.64
K	0.4552	0.1648	1.65	0.3501	0.04050	1.62
	0.4555	0.1649	1.65	0.3492	0.04040	1.62
	0.4537	0.1643	1.64	0.3509	0.04057	1.62

quired and therefore there is a considerable time saved by the use of sintered glass filter crucibles, which are highly recommended. We have used both the imported and domestic grades. Pyrex crucibles of medium porosity are quite satisfactory.

Magnesium oxyquinolate is highly insoluble in water and is precipitated for the most part in rather large granules which are easily washed and transferred. A limited amount of finely divided material with a tendency to "creep" is usually formed also but this offers no serious difficulty. The unusually low magnesium oxide conversion factor of 0.1157 suggests important obvious advantages.

In the drying of the precipitate to constant weight at 100–105° C. we have met with some disappointment. Contrary to the procedure usually cited in the literature we have found it necessary to heat for 2 hrs. and then for one or two $^1/_2$-hr. periods in order to attain constant weight. It may well be that the admixture of a small amount of sugar with the oxyquinolate precipitate considerably lengthens the drying period.

SUMMARY

1. A procedure for the assay of magnesium oxide in Solution of Citrate of Magnesia based on the oxine method has been described.

2. The results obtained by this procedure on ten commercial preparations of Solution of Citrate of Magnesia have been tabulated and compared with the corresponding results obtained by the U. S. P. method.

3. The oxine method of assay compares favorably with the U. S. P. method in the accuracy and reproducibility of results.

4. The oxine method represents a more simplified and rapid procedure and is therefore recommended.

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Celastrol, Spectrographic Characterization and Color Tests*

By L. F. Fieser and R. N. Jones

The ultraviolet absorption spectra (Fig. 1) were determined in ethanol solution and the log E molar values were calculated on the assumption of the formula $(C_{22}H_{30}O_3)_4 \cdot C_6H_{14}O$ for the celastrol sample and $C_{23}H_{32}O_3$ for methylcelastrol. Celastrol shows an intense, broad absorption band with a maximum at 422 mμ (log E = 3.84) and a shoulder at 256 mμ (log E = 3.8). The spectrum of methylcelastrol is similar, having a broad rounded band at 426 mμ (log E = 3.95) and a shoulder at 260 mμ (log E = 3.8).

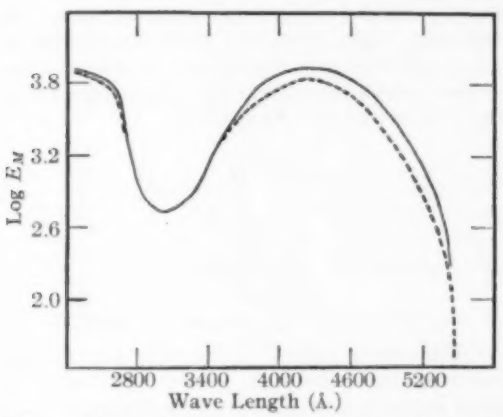

Fig. 1.—Absorption Spectra of Celastrol and Methylcelastrol in Ethanol.
- - - - - - - - Celastrol
———— Methylcelastrol

The absorption curves shown in Fig. 1 are wholly unlike those characteristic of α-naphthoquinone and its alkyl derivatives, but show one significant point of correspondence with the curves for substituted β-naphthoquinones. Cooke, Macbeth and Winzor (1) observed that a number of β-naphthoquinones show a rounded absorption band centering at 403–443 mμ, depending on the number and nature of the substituents, with log E values in the range of from 3.27 to 3.30 (see also Morton and Earlam (2)). Cooke, Macbeth and Winzor considered this distinctive band a characteristic mark of the β-naphthoquinonoid structure, but they did not emphasize the fact that many hydroxylated α-naphthoquinones exhibit a very similar band in the same region (3). Thus juglone (one OH) has a broad band at 425 mμ (log E = 3.7), isonaphthazarin (two OH groups) shows one at 445 mμ (log E = 3.3), and hydroxydroserone (three OH groups) has a maximum at 488 mμ (3.83). This broad band, which is completely absent from the spectrum of α-naphthoquinone (3), thus appears on the introduction of one hydroxyl group and is shifted progressively to longer wave lengths on increasing the number of such substituents. The distinctive band, however, is shown only by the free hydroxy compounds and disappears on replacement of the labile hydroxylic hydrogen atom. Thus acetylation of the hydroxyquinones results in a complete reversion to the α-naphthoquinonoid spectrum. The band shown by celastrol at 422 mμ thus might be indicative of either a β-naphthoquinonoid or a hydroxy-α-naphthoquinonoid structure, but the curve for the methyl ether provides a means of distinguishing between the two possibilities. The fact that with methylcelastrol the rounded band does not disappear but persists in essentially the original form affords a strong indication of the β-naphthoquinonoid character of the natural pigment. That the hydroxy compound and its methyl ether are so similar finds some analogy in the observation (1) that lapachol methyl ether has a spectrum in which the long wave length band is not so pronounced as in lapachol but is not affected to the same extent as in the acetate.

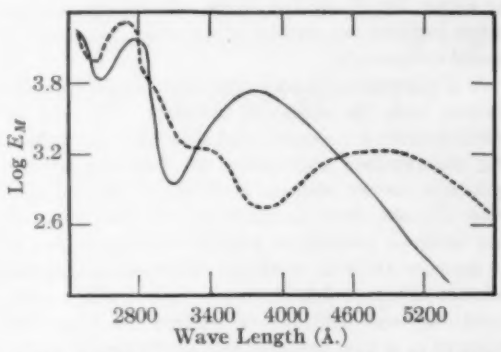

Fig. 2.—Absorption Spectra in Ethanol of 7-Hydroxy-1,2-naphthoquinone (Maxima in mμ and log E_{molar} Values: 270 (4.32); 300 (3.68 (Inflection)); 340 (3.23); 485 (3.22)) and 6-Hydroxy-1,2-naphthoquinone (278 (4.16); 366 (3.74)).
- - - - - - - - 7-Hydroxy-1,2-naphthoquinone
———— 6-Hydroxy-1,2-naphthoquinone

In the region of shorter wave length, the spectrum of celastrol departs considerably from that associated with such known β-naphthoquinones as have been studied (1, 3, 4), the chief point of difference being in the absence of a narrow band of intensity far superior to that of the broad long wave length band.

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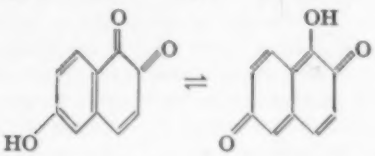
Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

Thus β-lapachone shows such a band at 256.5 mμ (log E = 4.45), together with shoulders at 282 mμ (3.98) and 333 mμ (3.24) (1), while celastrol shows only a low intensity shoulder at 256 mμ. The difference may possibly be associated with the presence of the hydroxyl group, and for comparison we examined the 6- and 7-hydroxy derivatives of 1,2-naphthoquinone (Fig. 2). Spectrographically, 7-hydroxy-1,2-naphthoquinone closely resembles β-lapachone, while the 6-isomer is so widely different as to suggest the possibility of a tautomerism:

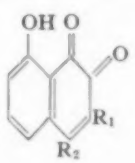


This is not without some analogy (5, 6). Clearly both compounds differ sufficiently from celastrol to warrant the conclusion that the pigment cannot be a 1,2-naphthoquinone having a hydroxyl group at either the 6- or 7-position. Unfortunately no other isomers are available for study as possible model compounds.

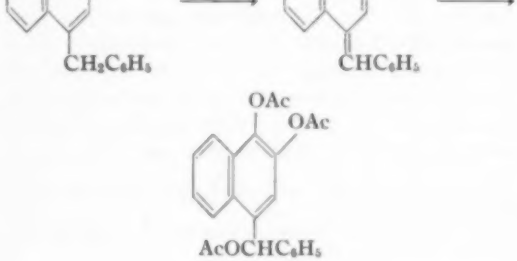
A β-naphthoquinonoid structure would be consistent with the colors of celastrol (red) and of methylcelastrol (orange), and also with the following observations concerning the behavior of the quinones toward sodium bisulfite. Although Gisvold (7) observed no reaction between celastrol and aqueous bisulfite, a prompt reaction occurs in an aqueous alcoholic medium. When an orange-red solution of celastrol in alcohol is treated with a saturated aqueous solution of sodium bisulfite, the pigment is at first precipitated but then rapidly dissolves to give a completely colorless solution. When a suspension of methylcelastrol is treated similarly with aqueous bisulfite solution the orange material soon dissolves to a colorless solution. On neutralization of the bisulfite with sodium carbonate the orange pigment is slowly reprecipitated. This characteristic behavior provides chemical evidence of an *ortho*-quinonoid structure.

Quinones having a hydroxyl group ortho to one of the quinone carbonyl groups give an intense coloration when treated with Dimroth's boroacetic anhydride reagent (5, 8), while other hydroxyquinones as a rule give no more color than when treated with acetic anhydride alone. Thus the Dimroth test is negative with 6- and 7-hydroxy-1,2-napthoquinone, while juglone (5-hydroxy-1,4-naphthoquinone) gives a positive orange-red test. Celastrol gives a light orange-yellow color with acetic anhydride alone, but on adding the Dimroth reagent a deep red color develops in the cold. This indicates that the hydroxyl group most probably is at the 8-position, although the 3-position may also be a possibility. It may be noted that the red color and high melting point (205° C.) of celastrol find some analogy in the properties of 6- and 7-hydroxy-1,2-naphthoquinone, which are orange-red and red, respectively, and melt at 165° C. and at 204° C. (9).

Another significant color test is that of Craven (10). Naphthoquinones having a free position in the quinonoid ring when treated in alcoholic solution with ethyl cyanoacetate and ammonia give an intense blue coloration, and the test is negative if both quinonoid positions are occupied by substituent groups. It has now been found that the test is applicable to *ortho* as well as *para* quinones. 2,6-Dimethyl-1,2-naphthoquinone and 4-benzyl-1,2-naphthoquinone both give intense blue solutions, while with the fully substituted β-lapachone the test is negative. With celastrol there is no sign of a blue color in the Craven test and instead an unusual phenomenon is observed. The substance dissolves in alcoholic ammonia with a deep reddish color and on adding ethyl cyanoacetate the color promptly fades and a colorless solution results. That the presence of a hydroxyl group does not interfere is shown by the observation that plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) gives an intense purple-indigo color in the Craven test. Furthermore, methylcelastrol gives a negative Craven test and undergoes no apparent change. It may be concluded that both the 3- and 4-positions are occupied by substituents, and the foregoing observations may be interpreted in terms of the following tentative formula for celastrol:



Confirmatory evidence is found in the observation that when celastrol is treated in the cold with acetic anhydride containing a trace of concentrated sulfuric acid (Thiele reagent) the initial yellow color slowly disappears and the solution becomes completely colorless. Among quinones other than those having a conjugated system with hydrogen on the terminal carbon atom (—CH=C—C=O), this behavior has been noted only with 4-alkyl-1,2-naphthoquinones, such as the 4-methyl and 4-benzyl derivatives (11, 12). With the benzyl compound the reaction has been shown to proceed through a tautomerism to the aci-form and a Thiele addition:



A similar reaction may be responsible for the bleaching of the color in the Craven test. It was noted also that on extracting celastrol from an orange ethereal solution with soda, acidifying the aqueous solution and extracting with ether, a pure yellow solution was obtained. This again suggests the tautomerism of a 4-alkyl-1,2-naphthoquinone.

From Gisvold's observations (13) it is evident that the alkyl groups R_1 and R_2 must both be fully saturated. An attractive speculation is that the group R_1 is methyl, for a methyl group occupying the same position is present in the natural naphthoquinone pigments plumbagin, phthiocol, hydroxydroserone and vitamins K_1 and K_2. In such a structure, furthermore, the methyl and hydroxyl groups would occupy the same relative positions as in plumbagin. The group R_2 which, in view of the optical activity of the pigment (14), appears to have a branched chain, may be a hydrogeranyl or a homohydrogeranyl group.

REFERENCES

(1) Cooke, R. G., Macbeth, A. K., and Winzor F. L., *J. Chem. Soc.* (1939), p. 878.

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(3) Macbeth, A. K., Price, J. R., and Winzor, F. L., *ibid.* (1935), p. 325.

(4) Goldschmidt, S., and Graef, F., *Ber.*, 61 (1928), 1858.

(5) Fieser, L. F., *J. Am. Chem. Soc.*, 51 (1929), 2471.

(6) Campbell, W. P., and Todd, D., *ibid.*, 62 (1940), 1287.

(7) Gisvold, O., JOUR. A. PH. A., 29 (1940), 432.

(8) Dimroth, O., and co-workers, *Ber.*, 54 (1921) 3020; *Ann.*, 446 (1926), 97, 123; 456 (1927), 177.

(9) Fieser, L. F., and Dunn, J. T., *J. Am. Chem. Soc.*, 59 (1937), 1016.

(10) Craven, R., *J. Chem. Soc.* (1931), p. 1605.

(11) Fieser, L. F., and Bradsher, C. K., *J. Am. Chem. Soc.*, 61 (1939), 417.

(12) Fieser, L. F., and Fieser, M., *ibid.*, 61 (1939), 596.

(13) Gisvold, O., JOUR. A. PH. A., 29 (1940), 12.

(14) Gisvold, O., *ibid.*, 28 (1939), 440.

U. S. Pharmacopoeial Convention

Statement of Income and Expense for the Second Year of the 1940–1950 Decennial Period from May 1, 1941, to April 30, 1942*

The accounts of the U. S. Pharmacopœial Convention, covering the year ending April 30, 1942, have been audited by R. G. Rankin and Company, Certified Public Accountants of New York and Washington. At the direction of the U. S. P. Board of Trustees, copies of the Statement of Income and Expense, and of the Statement of Cash Receipts and Disbursements as prepared by the auditors are attached, together with supplemental data giving further details with respect to disbursements, under the heading Revision and Research.

As would be expected, the sales of the U. S. P. and its Supplements during the past year have been lower than those of the previous year. With the expectancy of the appearance of a new pharmacopœia, sales would naturally be at low ebb. The decrease noted in the expense of printing and binding might naturally be expected to follow the decrease in sales over this period.

An increase in Administration expenses for the year is explained by extra clerical work, largely in connection with the revision of the Constitution and By-Laws, by a meeting of the Constitution and By-Laws Committee in Detroit, and by a meeting of Dean Rogers and Dr. Bunce of the Committee with the Board of Trustees in New York City, together with several meetings of the Board and the interest on bank loans and miscellaneous general expenses.

Convention expense increases are notably due to the publication and distribution of the Proceedings of the 1940 decennial meeting as noted under Printing and Binding, and the general expenses which include an honorarium of $500.00 to Secretary Warren and the expenses of the 1942 meeting at Cleveland including counsel fees for the year.

The additional detailed statement regarding Revision and Research expenses which is included with this report, readily explains the decrease in revision expenses and the slight increase in those classified as research, when compared to the similar statement covering the activity of the previous year of the decade.

For the U. S. P. Convention,
W. PAUL BRIGGS, *Treasurer*

For the U. S. P. Board of Trustees,
E. F. KELLY, *Chairman*
ADLEY B. NICHOLS, *Secretary*

*Copies of this statement may be obtained by writing to the Secretary, Adley B. Nichols, 43rd St. and Kingsessing Ave., Philadelphia, Pa.

U. S. PHARMACOPŒIAL CONVENTION, WASHINGTON, D. C.

STATEMENT OF INCOME AND EXPENSE FOR THE YEAR ENDED APRIL 30, 1942

Income			
Sale of Pharmacopœias			
Collections		$ 8,022.17	
Less: Decrease in Accounts Receivable			
April 30, 1941	$2,349.31		
April 30, 1942	1,066.71	1,282.60	$ 6,739.57
Sale of Reference Standards, A. M. A. Articles, etc.			
Collections		$ 4,141.25	
Add:			
Increase in Collections Pending Remittance to the Treasurer			
April 30, 1942	$ 957.04		
April 30, 1941	288.75	668.29	
Increase in Accounts Receivable			
April 30, 1942	$ 495.08		
April 30, 1941	270.46	224.62	5,034.16
Interest on Investments			
Collections		$ 1,502.50	
Less: Amortization of Bond Premiums		270.05	1,232.45
Use of U. S. Pharmacopœia Text by Others			30.00
Miscellaneous Income			171.10
Total Income			$13,207.28
Less:			
Expense			
Cost of Publications Sold			
Publication and Sales Expense	$3,466.52		
Less: Increase in Inventory			
April 30, 1942	$7,920.01		
April 30, 1941	5,056.86	2,863.15	$ 603.37
Administration		4,818.68	
Convention		3,150.87	
Revision		23,103.79	
Research		6,704.73	
		$38,381.44	
Less:			
Reimbursement of Petty Cash Expenditures Applicable to Prior Year	$ 833.28		
Furnishings and Equipment Capitalized (at 75% of cost)	31.00		
Stationery and Supplies Inventory Adjustment	383.02	1,247.30	37,134.14
Excess of Expense over Income			
For the Year ended April 30, 1942—Exhibit "A"			$23,926.86

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS (GENERAL ACCOUNT) FOR THE YEAR ENDED APRIL 30, 1942

Cash on Deposit, April 30, 1941			$ 7,792.17
Add:			
Receipts			
Sale of Pharmacopœias		$ 8,022.17	
Sale of Reference Standards, A. M. A. Articles, etc.		4,141.25	
Interest on Investments		1,502.50	
Use of U. S. Pharmacopœia Text by Others		30.00	
Return of Petty Cash to General Fund		1,000.00	
Increase in Bank Loan		35,000.00	
Miscellaneous Income		171.10	49,867.02
			$57,659.19

Less:

Disbursements

	Publication and Sales	Administration	Convention	Revision	Research	Total	
Printing	$ 431.85	$.....	$ 858.52	$.....	$.....	$ 1,290.37	
Binding	2,746.38	.....	.....	.....	.....	2,746.38	
General	288.29	609.58	1,986.57	1,737.50	.....	4,621.94	
Clerical	.....	1,116.35	83.50	7,053.19	305.76	8,558.80	
Meetings	.....	1,396.94	.....	3,521.83	1,242.90	6,160.86	
Supplies	.....	100.31	55.88	2,780.59	782.15	3,718.93	
Postage and Telegrams	.....	184.24	166.40	1,235.68	101.68	1,688.00	
Honoraria and Salaries	.....	1,000.00	.....	6,775.00	.....	7,775.00	
Compensation	.....	.....	.....	.....	4,273.05	4,273.05	
Interest on Bank Loans	.....	411.26	.....	.....	.....	411.26	
Total	$3,466.52	$4,818.68	$3,150.87	$23,103.79	$6,704.73		41,244.59
Cash on Deposit, April 30, 1942—Exhibit "A"							$16,414.60

A MORE DETAILED AND SUPPLEMENTARY FINANCIAL STATEMENT OF THE REVISION EXPENSES

REVISION—MEETINGS

Public Hearing on U. S. P. XII, Philadelphia, November, 1941	$2,136.23
Traveling, hotel and incidental expenses for conferences with subcommittee chairmen, subcommittee groups, Food and Drug officials and others in maintaining the revision program. This item includes numerous weekly conferences during the fall and winter, of several members of the Executive Committee, for the purpose of checking comments on proof, editing texts, etc.	1,385.60
	$3,521.83

REVISION—SUPPLIES

Binders for new volume of circulars, etc.	$ 112.17
Stationery for revision office	221.09
Mimeograph supplies (paper for circulars, etc., stencils, ink and maintenance of machine)	891.63
Purchase of reference standards (nicotinic acid, riboflavin, sulfanilamide, menadione, ouabain, thiamine hydrochloride, ergotoxine ethanesulfonate)	1,275.65
Miscellaneous expenses	280.05
	$2,780.59

REVISION—CLERICAL

Stenographic and clerical assistance, Chairman's office. This includes the services of Mrs. Beulah Tullar, who has been of very great assistance in preparing manuscript for the printer, reading proof in all its stages, preparing index, etc. Mrs. Tullar has assisted in this part of pharmacopœial work for several revisions and consequently her services are highly valuable	$7,053.19

REVISION—POSTAGE AND TELEGRAPH

Postage, Express, Telephone and Telegraph service, Chairman's office	$1,235.68

REVISION—GENERAL EXPENSES

Translating the "Pharmacopœia and Physician" series into Spanish	$ 238.95
Reprints on "Pharmacopœia and Physician" series	155.32
Typewriter	100.49
Contribution to the AMERICAN PHARMACEUTICAL ASSOCIATION toward "Pharmaceutical Abstracts"	1,000.00
Miscellaneous	242.74
	$1,737.50

REVISION—SALARIES AND HONORARIA

Salary, Revision Chairman, E. Fullerton Cook	$4,375.00
Salary, Executive Assistant to Chairman, Adley B. Nichols	2,400.00
	$6,775.00

RESEARCH

Most expenses of subcommittee and Advisory Board activity, incurred in the routine of preparing texts for U. S. P. XII are charged as Research.

Subcommittee	Meetings, Supplies, Postage, Clerical and General	Technical Assistance	
2—Biological Products	$ 26.08		
3—Biological Assays	32.50		
5—Botany and Pharmacognosy	94.38		
6—Proximate Assays	113.63		
7—Inorganic Chemicals	225.46	Dr. F. K. Bell[1]	$1,783.30
		Joseph M. White, III[1]	150.00
8—Organic Chemicals	627.19	Dr. Melvin Green[2]	300.00
		Miss Elinor Sackter[2]	1,612.50
12—Solutions, Spirits, etc.	11.91		
13—Cerates, Ointments, etc.	24.44		
15—Nomenclature	4.44		
Digitalis Study, many conferences, hearings, etc., leading to the preparation of new reference standard and development of the cat assay for U. S. P. XII	450.81	C. I. Bliss and W. J. Youden[3]	82.25
Meetings $314.95			
Clerical 102.11			
Supplies 33.75			
Posterior Pituitary Assay	53.81		
Hormone Advisory Board	24.85		
Blood Board	100.98		
Vitamin Board	104.05	Dr. O. L. Kline[4]	45.00
Anti-anemia Board	221.91		
Injections, U. S. P. XII	.....	Dr. Louis Gershenfeld[5]	300.00
Miscellaneous	315.24		
	$2,431.68		$4,273.05
			2,431.68
Total			$6,704.73

[1] Assistant to Chairman Krantz in checking and developing tests and assays of inorganic chemicals, gases and certain organic texts.

[2] Assistant to Chairman Beal in checking and developing tests and assays for many new organic drugs.

[3] Assistance as expert statisticians.

[4] Technical assistance in determining physical factors for U. S. P. Reference Cod Liver Oil No. 2, and preparing report for publication. (U. S. Department of Agriculture.)

[5] Assistance to Chairman Cook in the preparation of the chapter and monographs on injections for U. S. P. XII.

Books Received

To September 15, 1942

WARREN H. COLE and CHARLES B. PUESTOW, *First Aid, Surgical and Medical.* D. Appleton–Century Company, Inc., New York, 1942. xxiii + 351 pp.

J.-ULYSSE DEMERS, *Étude sur la Préparation et la Sterilisation de Solutés Injectables.* Charrier & Dugal, Limitée, Angle Saint-Roch et Saint-Vallier, Québec, 1942. 156 pp.

CLEVELAND PENDLETON HICKMAN, *Physiological Hygiene.* Revised edition. Prentice–Hall, Inc., New York, 1942. xxi + 482 pp. $3.25.

TOM JONES, *Detailing by Druggists.* Romaine Pierson Publishers, Inc., New York, 1942. 52 pp. $0.75.

WINFIELD SCOTT PUGH, *War Medicine, a Symposium.* Philosophical Library, Inc., 15 East 40th Street, New York. 565 pp. $7.50.

C. A. ROJAHN, *Preparacion de Productos Quimicos y Quimico-Farmaceuticos.* Trans. and amplified by FRANCISCO GIRAL. Editorial Atlante, S. A., Mexico, D. F., 1942. Two vols., xxxix + 1002 pp.

DOROTHY SUTHERLAND, *Do You Want to Be a Nurse?* Doubleday, Doran and Company, Inc., Garden City, N. Y., 1942. 186 pp. $2.00.